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Feeding deterrence in sponges. The role of toxicity, physical defenses, energetic contents, and life-history stage.

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Abstract

The toxic and deterrent properties of two sympatric Mediterranean sponges, *Crambe crambe* and *Dysidea avara*, were studied at three stages of their life cycle: larvae, rhagons (functional recruits), and adults. We surveyed potential predators of these stages in the field, and selected the benthic fish *Parablennius incognitus* for predation tests on the larvae and rhagons, and the sea urchin *Paracentrotus lividus* for grazing experiments on artificial food containing adult sponge material. Larvae and one-week-old rhagons of *C. crambe* were readily eaten by the fish, while two-week-old rhagons were not. Larvae and rhagons of *D. avara* were not eaten by fish, and even larvae stained bright red to make them more conspicuous were rejected. Artificial food containing fresh *C. crambe* material, natural doses of its crude extract, or extracted sponge material were not grazed by *P. lividus*. All these preparations were grazed at the same rate as the controls in the case of *D. avara*. A deterrent effect was obtained in food containing double the natural concentration of avarol, the main active metabolite of *D. avara*. The energetic content of *C. crambe* is significantly higher than that of *D. avara*. Thus, adult stages of the energy-rich species are better protected from predation than those of the energy-poor species, by both physical structures and chemical molecules. It is concluded that contrasting defensive strategies can be displayed at different stages of the life cycle. No dichotomy was found between chemical and physical (spicules, tough organic structures) defenses: the species defended chemically as an adult (*C. crambe*) also featured physical defenses, while in the less chemically deterrent species (*D. avara*) structural, tough materials were much more scarce. The need to seek suitable test organisms at each life-history stage to understand the role and importance of defense mechanisms in the species' survival is stressed.

Keywords: Energetic contents; Feeding deterrence; Mediterranean sea; Physical defenses; Sponges; Toxicity; Unpalatability

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1. Introduction

Benthic sessile species are often defended against predators by the production of physical structures and/or chemical substances (Pennings and Paul, 1992; Van Alstyne et al., 1994). The anti-predatory role of numerous bioactive compounds has been documented in laboratory and field assays (e.g. Hay and Fenical, 1988; Paul and Van Alstyne, 1988; Pawlik et al., 1988; Rogers and Paul, 1991; Hay, 1992; Hay et al., 1994; McClintock et al., 1994; Pawlik et al., 1995), and contrasting results have been obtained depending on the potential predator used to evaluate anti-predatory effects (Hay and Fenical, 1988; Pennings et al., 1994). Chemical unpalatability is another decisive factor not necessarily associated with toxicity (Schulte and Bakus, 1992; Pawlik et al., 1995). Thus, unpalatable metabolites, apparently innocuous in laboratory screenings, may be more effective in deterring predation than toxic substances.

The anti-predation role of physical structures such as calcium carbonate in seaweeds (Pennings and Paul, 1992; Pennings and Svedberg, 1993; Hay et al., 1994) or sclerites in invertebrates (Harvell et al., 1988; Harvell and Fenical, 1989; Van Alstyne and Paul, 1992; Van Alstyne et al., 1992; Chanas and Pawlik, 1995) has also been increasingly studied. Food quality is another factor that has been incorporated to the study of anti-predatory effects (Berenbaum, 1995). Hay et al. (1994) suggested that the combined effects of toxins (chemical defense), calcification (physical defense) and food quality may be greater than the sum of their separate effects in seaweed defense. The study of the relationship between food quality and chemical deterrence in sponges has been approached both by experimental manipulation (Duffy and Paul, 1992; Pennings et al., 1994) and by correlational studies between energy contents and chemical deterrence (McClintock, 1987; Chanas and Pawlik, 1995). Chanas and Pawlik (1995) found no nutritional difference (except for the amount of lipids) between palatable and chemically defended sponges. In contrast, Duffy and Paul (1992) and Pennings et al. (1994) found that chemical defenses were less effective in high- than in low-quality artificial foods. These contrasting results are not contradictory but rather may indicate species-specific patterns.

In general, the more is known about the underlying mechanisms of chemical defense in marine environments, the more complicated and species-specific the processes appear (Meyer et al., 1994). Chemical variation throughout the species' biological cycle (Turon et al., 1996) as well as the role that physical structures or energy constraints may play in the observed lack of predation must also be ascertained. In particular, knowledge of feeding deterrence at different stages of the life-history of benthic sessile invertebrates is scarce (Lindquist et al., 1992), and it is completely missing in sponges.

The two species chosen for this study, *Crambe crambe* (Schmidt) and *Dysidea avara* (Schmidt) are among the most widespread littoral sponges in the western Mediterranean (Uriz et al., 1992a). They display contrasting life-history strategies (authors' current research), as exemplified by the different efficiency in particle retention (high and low clearance rates, respectively), the different turnover rates (slow and fast-growing strategies, respectively), as well as presence (*C. crambe*) or absence (*D. avara*) of siliceous spicules. *C. crambe* produces an array of toxic metabolites, some of them still undetermined (Berlinck et al., 1990; Jares-Erijman et al., 1991; Berlinck et al., 1992),

which show strong activities against marine bacteria, bryozoan larvae, and sea-urchin embryos (Becerro, 1994; Becerro et al., 1994, 1995). The main secondary metabolites of *D. avara* are avarol and its quinone avarone (Minale et al., 1974). Both metabolites display antimitotic activities (Cariello et al., 1980; Martín and Uriz, 1993), inhibit the fixation and metamorphosis of the larvae of the bryozoan *Bugula neritina* (Martín and Uriz, 1993) and show antibacterial, as well as antimitotic and cytostatic activities against pathogenic microbes and tumour cells, respectively (Müller et al., 1985; Seibert et al., 1985; Uriz et al., 1992b). A third active metabolite has also been described from this species (avarol monoacetate) but it may be the result of chemical manipulation (Crispino et al., 1989). The deterrent effect of a mixture of avarol/isoavarol obtained from the Pacific sponge *Dysidea* sp. has already been assayed against some fish and crabs (Pennings et al., 1994). The latter study showed that these metabolites usually deterred fish but not crabs from feeding when mixed with commercial fish food at concentrations of 2% and 4%. In contrast, reef fish were not deterred from predation on squid pieces coated and injected with solutions of avarol/isoavarol at the same concentrations.

In this paper we compare the deterrent effects of those two sympatric sponges at three different stages of development: free larvae, recruits with functional aquiferous system (rhagons), and adults. Besides, we assay the role of toxic compounds in deterring predation versus other potential defenses in adult sponges (e.g. siliceous spicules and tough structures such as foreign materials, collagen or spongin fibres). We also evaluate the nutritional quality (in terms of energy contents) of both species. By performing this study, we aimed to ascertain feeding deterrence at different life-history stages. Before selecting the assay organisms, the potential consumers of larvae and adult stages of the sponges in the study area, as well as signs of predation on adult individuals, were investigated.

2. Material and methods

2.1. Choice and collection of potential predators

Potential predators of sponges' larvae and recruits were sought among small benthic fish of the family Blenniidae which shared habitat with the sponges studied and fed on small mobile organisms of the size range of the sponge larvae (in situ observations). *Parablennius incognitus* (Bath) and *Tripterygion tripteronotus* (Risso) were two of the most abundant species in the area studied (Macpherson, 1994) and were therefore chosen for the experiment. Individuals (3-4 cm in length) of both species were netted in the locality of Blanes (NE of Spain) in early summer, 1995 and put in aquaria for acclimation. They were collected from the same habitat as the sponges studied. They were fed a ground mixture of fish and shrimps until they were completely acclimated to feeding in the aquarium as assessed by voracious consumption of the food supplied. Later, they were starved for three days. *T. tripteronotus* was always restless and frequently died. *P. incognitus* was well adapted, fed actively and showed alert behaviour. Thus, we used the latter species, to avoid artefacts in the experiment due to fish stress.

For the experiment on adult sponges, preliminary evaluation of the potential

consumers in the area of study was performed. The most significant of these, in terms of biomass, were echinoderms. Thus, the number of echinoids and asteroids found in ten 5×1 m horizontal transects in the same zone where the species were sampled were counted. *Paracentrotus lividus* (Lamarck) was by far the most abundant echinoid species and was used in the experiments. Individuals of *P. lividus*, of approximately the same size (5–6 cm in diameter), were collected for the experiments in the same locality as the adult sponges.

2.2. Sponge collection and handling

For the experiments with adults, six specimens of *C. crambe* and six of *D. avara* were collected by SCUBA diving near Blanes (NE of Spain) at depths of between 3 and 10 m in summer, 1994. They were separately introduced into plastic bags underwater and transported to the laboratory. A further set of nine specimens of *D. avara* were collected for a second experiment in summer, 1995 (see below). Once in the laboratory, the sponges were frozen and freeze-dried. All handling and treatment of the samples was performed separately to ensure independent replicates.

Abundant free larvae were obtained in the laboratory from mature specimens of both species collected at the locality of Blanes in the summer of 1995. Living specimens containing mature larvae were squeezed under a stereomicroscope, and the larvae released were stimulated to swim by stirring the water for a few seconds with a pipette. Swimming larvae with healthy aspect were collected by means of a pipette and used for settlement and feeding experiments.

Between 80 and 100 larvae of each species were placed in 8 to 10 Petri dishes (10 larvae in each dish) with filtered sea water in the dark at 22°C. Settlers of both species were obtained two days after release of larvae.

2.3. Extraction procedure

For the grazing experiment, the adult specimens of *C. crambe* were extracted three times (5, 10, and 15 min) with dichloromethane (DCM), which has proved to extract all the toxic molecules of this species (Becerro, 1994). The solvent was evaporated under reduced pressure and the residue was re-dissolved in 2 ml of acetone and added to the artificial food (see below). Acetone was used to ensure dissolution in the aqueous agar medium. As for *D. avara*, the sponges used were extracted with acetone (which extracts avarol and the other toxic metabolites very efficiently) three times (5, 10, and 15 min), the solvent was then evaporated and the residue was re-dissolved in 2 ml of acetone. Pure avarol was also obtained in crystalline form from fresh sponges by chemical extraction and successive fractionation following the procedure of Minale et al. (1974).

2.4. Artificial food preparation

Since *P. lividus* feeds preferentially upon Phaeophyceae in the field (43% of the diet, Verlaque, 1987), and especially on the *Cystoseira* spp. assemblages (Verlaque and Nédelec, 1983; Verlaque, 1984), the brown alga *Cystoseira mediterranea* Sauvageau was

chosen as the basis of artificial food for a grazing experiment. This alga was also collected, on the day of the bioassays, from the shallow sublittoral of Blanes (at a maximum depth of 1 m). Only the upper parts of the thalli were used.

The algae, previously blotted on paper towels, were ground in a mincing machine. Ten grams of freshly ground algae was added to 60 ml of an agar solution (5% in distilled water) at 50°C and mixed. A Petri dish was used as a mould to obtain disk-shaped plates of food. A piece of stainless steel was placed on the base of the agar plates to give negative buoyancy. The different treatments were added to this mixture when the temperature fell to 45°C.

2.5. Predation on adult individuals

A method based on Steinberg and Van Altena (1992) was used to test the effects of chemical and physical structures on the feeding of *P. lividus*. The sea urchins were placed in separate aerated aquaria (5 l) with filtered sea water (FSW) at constant temperature (18°C). They were starved for periods of 1 week for the first experiment with *C. crambe* and *D. avara*. In a second, longer experiment with *D. avara* (see below), sea-urchins were starved for two weeks. In all experiments, the FSW was changed daily to prevent proliferation of micro-organisms.

In the first experiment, five treatments were set up: (1) control-1, with artificial food composed of *C. mediterranea* extracted with DCM (*C. crambe* experiment) or acetone (*D. avara* experiment); (2) control-2, with *C. mediterranea* alone; (3) extract, in which the 2 ml of acetone with the extract obtained from each sponge was added to the artificial food; (4) extracted sponge, in which the sponge material remaining after extraction was added to the artificial food, and (5) untreated sponge, in which ground fresh sponge material was added to the food. We prepared three replicates of control-1 and five replicates of the remaining treatments.

To ensure that the concentration of extracts and other sponge materials was approximately the same as that found in nature, the sponge samples used for extraction and for the unextracted sponge treatment had approximately the same surface area and thickness as the artificial food plates (20 cm^2 and 7 mm, respectively). To avoid a possible effect due to the solvent, 2 ml of acetone was also added to the control plates and to the treatments other than the extract treatment. Moreover, six artificial food plates were prepared with *C. mediterranea* extracted with DCM and with acetone (three replicates of each) to make sure that no effect due to chemical extraction *per se* could affect the feeding behaviour of the sea urchins.

The artificial food was weighed and offered to the starved sea urchins. Twenty urchins were used for each sponge species and each was treated separately. The experiment was stopped after 48 h. During this period, aquaria received the same handling as during starvation. After 48 h the agar plates were drained and blotted with filter paper, and their weight losses were recorded.

After examination of the results of this first experiment, we performed a second experiment with *D. avara* in which we studied the effect of different avarol concentrations and whether the feeding behaviour of the sea urchin changed over time. The treatments consisted of the addition of two different amounts of purified avarol

(dissolved in 2 ml of acetone) to the artificial food. The resulting concentrations in the agar plates were similar to (0.2% fresh weight) and double (0.4% fresh weight) that found in the sponge in nature (Minale et al., 1974). Controls consisted of artificial food with algae alone, to which 2 ml of acetone was added. Three runs of these three treatments with three replicates each were conducted simultaneously, giving a total of 27 experimental units and 27 sea urchins. One of the three sets of treatments was weighed after 24 h, another after 48, and the third one after 72 h. Thus, we conducted an experiment with two factors: treatment (three levels: control, 0.2% avarol, and 0.4% avarol) and time (three levels: 24, 48, and 72 h). The aquaria handling was the same as for the previous experiment.

In both experiments, in order to check for autogenic changes in the weight of the agar plates, control plates ($n = 5$ and $n = 3$, respectively) were introduced into aquaria in the same conditions and for the same periods as the treatments but without sea urchins. There was a slight gain of weight in these plates, attributable to the hydration of the material. In fact, agar plates gained weight in the first 24 h and then remained approximately constant. The weight gains were 0.38 ± 0.127, 0.39 ± 0.146, and 0.37 ± 0.125 g (mean and SE) after 24, 48, and 72 h, respectively, which represent about 1.8% of the initial weight. Final weights in the experimental plates were adjusted accordingly.

In all cases, the sea urchins used in the experiments were subsequently dried (48 h at 100°C) and weighed. The variable analysed was the wet weight of food consumed (in g) per dry weight (g) of sea urchin.

2.6. Predation on free larvae and rhagons

In the first experiment five free larvae (which are bright red) of *C. crambe* were offered to each of seven specimens of *P. incognitus*, previously starved for three days, by slowly releasing them within the visual field of the fish. The same procedure was followed with free larvae of *D. avara* offered to seven fish (five larvae each). The number of larvae eaten by each fish was recorded after 30 s, and for the next two h fish were monitored to check for regurgitation of the larvae.

Since the fish seemed to ignore the presence of the larvae of *D. avara*, we attempted to determine whether they were inconspicuous to the fish due to its white colour. We therefore repeated the experiment (seven fish, five larvae each) with larvae coloured with a red stain (E-122) used in baking. Common fish food (consisting of a ground mixture of fish and shrimps) was also stained to control the influence of the stain on the feeding behaviour.

In a second phase, five larvae of *C. crambe* were offered to each of ten fish that had been fed the day before.

To determine the anti-predatory effects of newly settled sponges we used one- and two-week-old rhagons. The latter had developed an inhibition ring around them (Fig. 1) in the case of *C. crambe*, but not in *D. avara* (Fig. 2). We offered three one-week-old rhagons of each sponge species, scraped from their settlement surfaces, to each of seven starved fish. The same procedure (seven fish, three rhagons each) was repeated with two-week-old rhagons of both species. The number of rhagons either eaten or nibbled

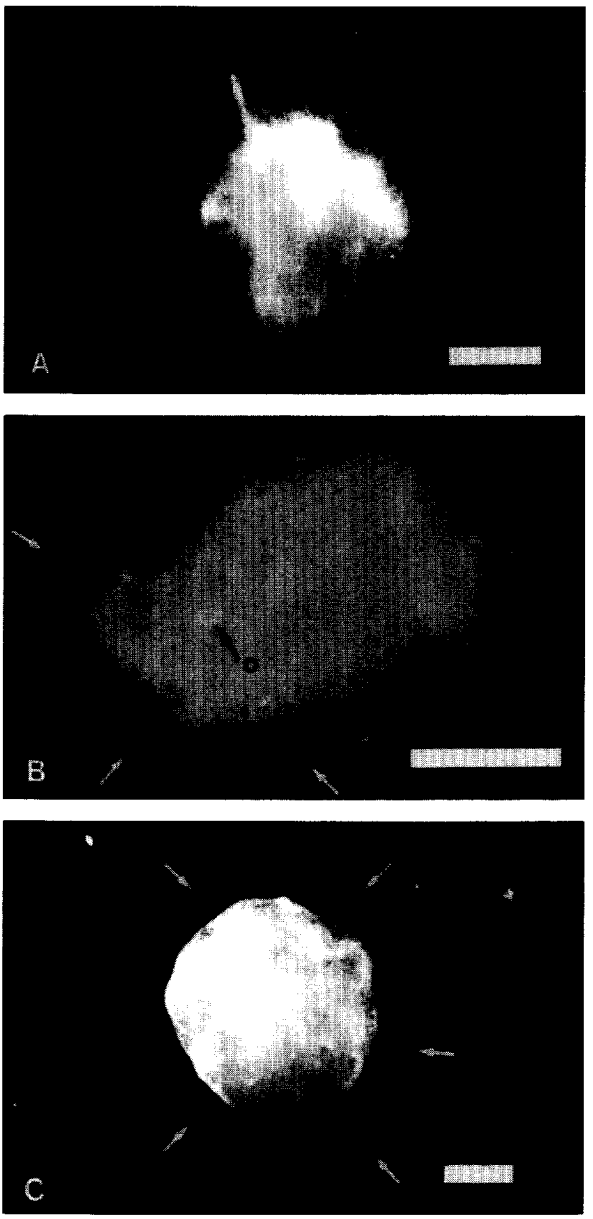

Fig. 1. Rhagons of *Crambe crambe*: A, one-week-old individual; B, ten-day-old individual with an osculiferous process (o) at the beginning of the formation of the inhibition halo (arrows); C, two-week-old individual, note the well-developed inhibition ring (arrows). Scale bar: 0.5 mm.

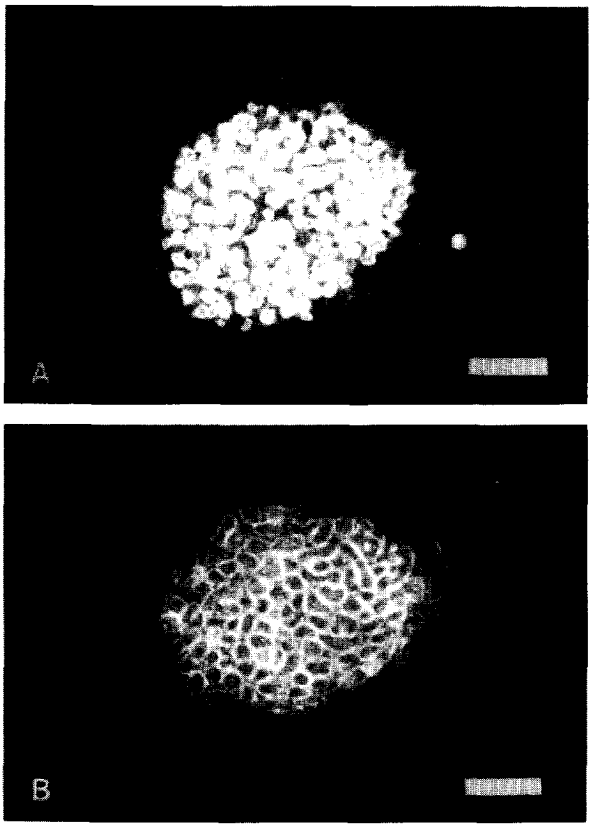

Fig. 2. Rhagons of *Dysidea avara*: A, one-week-old individual; B, two-week-old specimen. The oval profiles inside the rhagons are canals of the aquiferous system. Scale bar: 0.5 mm.

and then rejected was recorded after 30 and 60 s. Fish were monitored for 2 h to detect eventual regurgitation of rhagons. For the two-week-old rhagons we also put one Petri dish containing seven living rhagons fixed on the bottom in each of 3 aquaria containing 7 fish each, and left it there for 48 h. The procedure was followed for both species. After this time, the number of rhagons still present was recorded and they were examined under a stereo-microscope for the presence of bites.

2.7. Energy content

The energy contents of *C. mediterranea* were calculated and compared to those obtained for *C. crambe* and *D. avara*, to determine the role of energy constraints in predation. Two specimens of each species were collected, cleaned of any foreign material and endobionts (barnacles, polychaete tubes, snails, etc.), dried, weighed, ground and compressed into a pellet with a Parr Pellet Press. These pellets were freeze-dried and re-weighed before they were burned in a 1261 Isoperibol Bomb

Calorimeter (Parr Instrument Company). After preliminary tests, 200 mg (dry weight) pellets were selected as the most suitable size. The caloric value of the sample is the number of heat units liberated by a unit mass of sample when burned with oxygen in an enclosure of constant volume, and is given in $J \cdot mg^{-1}$.

2.8. Statistical analyses

In the first experiment on *C. crambe* and *D. avara*, the data (g consumed/g sea urchin) were analysed by one-way ANOVA after rank transformation (Potvin and Roff, 1993) to meet the assumptions of this parametric test. Post-hoc tests were made by the Tukey procedure (Zar, 1984).

Data from the second experiment on *D. avara* complied with the assumptions of homoscedasticity and normality (Barlett and Kolmogorov–Smirnov tests, respectively —Zar, 1984). A two-way ANOVA was therefore performed on the raw data, the factors being treatment (three levels) and time (three levels). Whenever one main factor was found to be significant in the overall ANOVA, pairwise comparisons (Tukey test) were made between levels of this factor across levels of the other. The energy value of the three species was compared by one-way ANOVA and post-hoc Tukey tests. No transformation of these data was necessary.

No analyses were necessary for the experiments with larvae and rhagons since the results were consistently all or nothing (either 100% eaten or 100% rejected) and no variance could therefore be associated with the treatments.

3. Results

3.1. Potential consumers

In the ten transects surveyed, three echinoid species were found: *Paracentrotus lividus* (Lamark), density: 2.8 ± 0.39 ind/m^2 (mean $\pm$ SE), *Sphaerechinus granularis* (Lamarck): 0.4 ± 0.09 ind/m^2, and *Arbacia lixula* (L.): 0.3 ± 0.09 ind/m^2. Sea stars were rarely found: 13 *Echinaster sepositus* (Retzius) and one *Marthasterias glacialis* (L.). Occasional signs of grazing were observed on *D. avara* but never on *C. crambe*. Small fish of the family Blenniidae were abundant in the rocky walls where the sponges live. Among them, the species *Parablennius incognitus* (Bath) and *Trypterigion trypteronotus* (Risso) were particularly well represented (Macpherson, 1994).

3.2. Predation on adults of C. crambe and D. avara

Analysis of variance of the data obtained in the experiment with *C. crambe* (Table 1) showed a significant treatment effect ($p = 0.001$), which was exclusively due to the amount of food eaten in the controls (Tukey test, $p < 0.01$ in any case when control-1 or control-2 were compared with the remaining treatments). Toxic compounds alone, untreated sponge material, and extracted sponge material strongly deterred predation of the sea urchin when compared with controls after 48 h (Fig. 3A). No significant

Table 1
One-way ANOVA for effect of treatment (control, fresh sponge, physical structures, and toxic compounds) on the amount of artificial food grazed per gram of sea urchin. (Data were rank-transformed prior to analysis)

	Source	SS	DF	MS	F	p
C. crambe	Treatment	601.500	4	150.375	6.994	0.0014
	Error	387.000	18	21.500		
D. avara	Treatment	112.975	4	28.243	0.661	0.651
	Error	768.525	18	42.695		

difference was detected among these three treatments ($p > 0.99$ in all pairwise comparisons).

In the experiment with *D. avara*, in contrast, the ANOVA results (Table 1) showed no significant treatment effect ($p = 0.65$). The consumption of food in the five treatments was similar (Fig. 3B).

As the plates in which the alga was extracted with DCM or acetone (control-1) featured consumption rates similar to the unextracted control plates (control-2, Fig. 3), an effect of the extraction procedure alone can be ruled out.

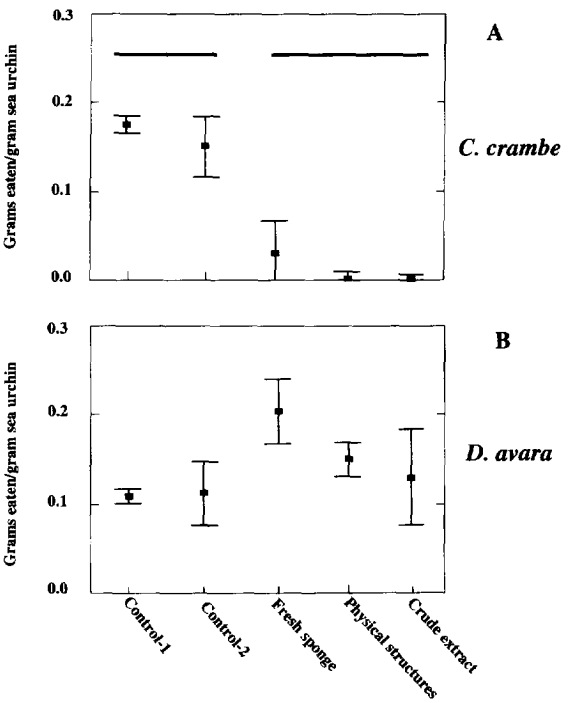

Fig. 3. Grams of artificial food consumed per gram of sea urchin in the grazing experiments with (A) *Crambe crambe* and (B) *Dysidea avara*. Control-1 corresponds to *C. mediterranea* extracted with dichloromethane (A) or acetone (B). Control-2 corresponds to non-extracted *C. mediterranea*. Vertical bars are standard errors. Horizontal bars join treatments whose means proved not different ($p < 0.01$) in a Tukey test .

In view of the lack of deterrence of *D. avara* extracts, previously reported as being highly toxic (see Introduction), we performed a second experiment in which the time of exposure to and concentration of toxic chemicals were taken into account. The results of the two-factor ANOVA (Table 2) indicated a significant concentration effect ($p = 0.016$), while neither the time factor ($p = 0.23$) nor the interaction ($p = 0.44$) proved to be significant. Post-hoc comparisons (Tukey test) were therefore performed between the concentration levels across the time levels. There was no significant difference between the control and the treatment with avarol at 0.2% ($p = 0.56$), while the treatment with avarol 0.4% showed a significant decrease in consumption with respect to control ($p = 0.01$) (Fig. 4).

3.3. Collection of rhagons

Two days after release, 50% of the larvae of *C. crambe* and 40% of those of *D. avara* had settled. One week after settlement, postlarvae of *C. crambe* were flattened, without conspicuous oscula (Fig. 1A). From ten to twelve days after settlement, recruits of *C. crambe* displayed the characteristic rhagon form (Uriz, 1982): hemispherical to flattened spots, 1 to 1.5 mm in diameter, with one or several osculiferous chimneys open. The rhagons were surrounded by a 0.5–2 mm wide ring devoid of bacteria, diatoms or ciliates, which invaded the remaining surface of the Petri dish (Fig. 1B,C). We interpreted this halo as a result of the toxic and antimicrobial activities of the species (Martín and Uriz, 1993; Becerro et al., 1994). Rhagons of *D. avara* were somewhat more flattened and larger (up to 4 mm in diameter), and showed a different morphology, with the central part full of subspherical canals (Fig. 2A,B). Two-week-old rhagons were not surrounded by any inhibition halo (Fig. 2B). One month later, rhagons of both species, maintained in aquaria, produced healthy juvenile sponges.

3.4. Predation on larvae and rhagons

The results of this experiment are summarized in Table 3. Fish were not deterred from feeding on the larvae of *C. crambe*. All the fish (starved and fed the day before) fed on the five larvae offered to them. They also ate all the scraped one-week-old rhagons. No rejection of larvae or rhagons by any fish was detected during the 2 h following consumption. In contrast, two-week-old rhagons scraped from the substratum were consistently nibbled, but quickly rejected, by the fish. The same rhagon was tried up to three times by the same fish. Moreover, no predation sign was observed in the

Table 2
Two-way ANOVA for treatment (control, 0.2% avarol, and 0.4% avarol) and time (24 h, 48, and 72 h) effects on artificial food grazed per gram of sea urchin

Source	SS	DF	MS	*F*	*p*
Treatment	0.0435	2	0.0218	5.4220	0.0169
Time	0.0132	2	0.0066	1.6403	0.2268
Treatment · time	0.0160	4	0.0040	0.9980	0.4389
Error	0.0602	15	0.0040		

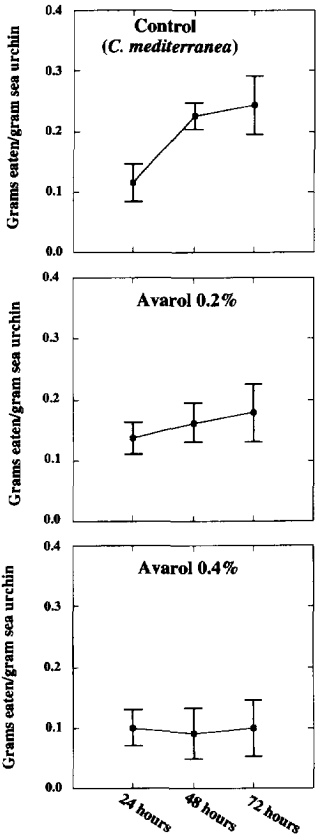

Fig. 4. Grams of artificial food consumed per gram of sea urchin as a function of time and concentration of avarol in the food. Bars are standard errors.

Table 3
Results of the deterrence experiments on larvae and rhagons of the two sponge species

Treatment	No. of fishes	No. of rhagons or larvae	% eaten *C. crambe*	% eaten *D. avara*
Non manipulated larvae starved fishes	7	35	100	0
Non manipulated larvae non starved fishes	10	50	100	-
Stained larvae starved fishes	7	35	–	0
Scraped 1-week-old rhagons	7	21	100	0
Scraped 2-weeks-old rhagons	7	21	0	0
Non scraped 2-weeks-old rhagons	21	21	0	0

two-week-old rhagons adhering to the substratum after 48 h. Consequently, larvae and young (one-week-old) recruits of *C. crambe* featured no toxicity or unpalatability, which nevertheless appeared in two-week-old recruits. This may be related to the differentiation from archeocytes of the first spherulous cells, which seem to be responsible for sponge toxicity (Uriz et al., 1996).

Free larvae of *D. avara* were consistently not eaten by *P. incognitus* . Unstained larvae were apparently not detected by fish, but red-stained larvae were detected, nibbled and rejected by fish in all instances. Fish food, red-stained, was quickly eaten by the fish, so the unpalatable effect could not be attributed to the stain.

Rhagons of *D. avara*, either scraped from the substratum (one- and two-week-old) or fixed to it (two-week-old) were not eaten in any instance.

Whether rhagons of the two sponges studied produced toxic chemicals could not be ascertained in this study due to the large amounts of individuals necessary to perform chemical or toxicological analyses.

3.5. Energy content

The sponge *C. crambe* featured caloric values of 14.35 ± 1.43 (mean $\pm$ SE) $J \cdot mg^{-1}$, the sponge *D. avara* 8.59 ± 1.07 $J \cdot mg^{-1}$, and the algae *Cystoseira mediterranea* 9.17 ± 0.61 $J \cdot mg^{-1}$ of sponge dry weight. One-way ANOVA detected significant differences in the energy content of these three species ($p = 0.001$), the values of *C. crambe* being significantly higher than those of *D. avara* ($p = 0.0023$) and *C. mediterranea* ($p = 0.003$) (Tukey test). Thus, the species with the highest caloric value is also the best protected from predation by means of both chemical compounds and physical structures (siliceous spicules and collagen fibrils).

4. Discussion

The results support the idea of *C. crambe* as a sponge with anti-predatory properties. This agrees well with the lack of signs of predation on adult specimens in the field. Chemical feeding deterrence contributes to the lack of predation on this species, but it does not seem to be the only relevant factor. The sea urchin *P. lividus* failed to feed on one of its habitual foods (the brown algae *C. mediterranea*) on agar plates containing natural concentrations of the sponge extracts. In addition, *P. lividus* was also deterred when the algae were mixed with exhaustively extracted sponge material. Toughness due to spicules and collagen may account for the inhibitory effect of this treatment. Spicular contents and toughness have been shown to affect herbivory (Van Alstyne and Paul, 1992; Pennings and Paul, 1992; Hay et al., 1994), but no differences in toughness between palatable and chemically deterrent sponges were found by Chanas and Pawlik (1995). In other Mediterranean localities (e.g. Medes Islands, NE of Spain) other fish species, such as the Sparid *Diplodus puntazzo* (Gmelin) usually feeds on sponges with high content in spicules and collagen (e. g. *Cliona viridis* (Schmidt)) but it has not been found to feed on *C. crambe* (unpublished results), which lives in the same habitat (i.e. moderately illuminated seaweed assemblages).

Of course, compounds insoluble in dichloromethane (i.e. not extracted) may also have been responsible for this deterrent effect. However, when *C. crambe* that had been extracted with DCM was re-extracted with several solvents, no toxic properties were found in tests with a variety of organisms (Becerro, 1994). DCM seems, therefore, to take up all the toxic compounds in this species. This does not guarantee, however, that the compounds remaining in the extracted sponge are not ecologically relevant (e.g., they may be non-toxic, but unpalatable). On the other hand, although the toxic compounds are not the sole anti-predatory defense against the grazer *P. lividus*, their role may be important against other potential consumers. This would explain why the two-week-old rhagons are protected from predation by *P. incognitus*. As they feature only a few spicules and little collagen, chemical rather than physical defenses may be responsible for the lack of predation.

Larvae of *C. crambe* are not protected from predation. They do not seem to be toxic (in the short term) or unpalatable, since fish always fed on them and did not reject them. These larvae would be preferentially consumed, due to their size, by small benthic fish such as *P. incognitus* and other Blenniidae, which are most active during the period of larval release and settlement of this sponge (Macpherson, pers. comm.). Thus, only the sessile stages (from two-week-old rhagons to adults) of this species seem to be protected from predation. Protection of the mobile stage may not be decisive for survival since this species has high reproductive rates (Uriz et al., 1995) and displays a relatively long period of larvae production (July and August in the area of study). Protection of sessile stages may be more relevant for a species with extraordinarily low growth rates such as *C. crambe*: even small sponges between 10 and 200 mm^2 need about a year to double their size (authors' unpublished results).

In the case of *D avara*, the presence of the crude extract did not deter feeding by *P. lividus*, nor did the presence of fresh sponge or extracted (i.e., non toxic) sponge. The latter two, if anything, resulted in increased consumption rates. The treatment with avarol at natural concentration featured similar levels of consumption as control, and a deterrent effect was displayed only when the sea urchins were fed on artificial food containing doses of avarol double those naturally occurring in the sponge. Even in this last case, starved sea urchins consumed similar amounts of food from all treatments in 24 h, the deterrence being noticeable only after 48 and 72 h. There might thus be a role of avarol and its derivatives in preventing predation, but this effect seems rather weak, at least at natural concentrations. However, deterrence may occur in more natural conditions, when sea urchins are not starved and can choose among a variety of foods. Our experiment was a non-choice one (the urchins could either eat or starve), because we wanted to study potential deterrence with respect to controls and isolate it from all other factors. On the other hand, tough, structural elements are scarce in this species, thus making any physical defense mechanism unlikely, in accordance with the fact that extracted sponge did not deter feeding of *P. lividus* on the artificial food.

The very slow increase with time of the food consumed in the treatment with avarol at natural concentration (Fig. 4) seems to indicate that sea urchins can ingest only moderate doses of toxic compounds, thereby avoiding strong detrimental effects. This behaviour has been described for other generalist herbivores feeding on various toxic organisms (Lindquist and Hay, 1995). These predators may produce enzymes that allow

them to consume small quantities of noxious compounds with minimal detrimental effects (Brattsten, 1992).

Larvae of *D. avara* were not eaten by *P. incognitus*. They may not have been visible to the fish because they were detected, eaten and rejected only after being stained in red (fish fed normally on artificial food with the same stain). Thus, larvae of *D. avara* seem to be unpalatable for *P. incognitus* and are protected from predation even if they are detected. The factor responsible for this unpalatability remains unknown.

Rhagons of *D. avara* were not eaten. They may not have been attractive to fish (they are almost translucent), but they may already have produced avarol. A cryptic appearance due to translucency can protect larvae and rhagons of sponges from predation like many planktonic organisms, although in the case of *D. avara* they also seem to be protected by deterrent substances. Avarol is contained in the choanocytes in this species (authors' current research), and these cells are necessarily present in the functional rhagons. Avarol, whose deterrent effect on *P. lividus* was not evident during the first two days of the grazing experiment, may be more effective as a deterrent of predation on *D. avara* by fish. Pennings et al. (1994) found a deterrent effect against diverse consumers of avarol/isoavarol at doses higher (2% and 4% dry weight) than in our study, when mixed with high quality food.

As for the relationship between energy contents and predation, no energetic constraints seem to be responsible for the lack of predation on *C. crambe*, as would be predicted from the arguments of Duffy and Paul (1992). *C. crambe* has higher caloric contents than *D. avara* and *C. mediterranea*, which feature similar values. In fact, the caloric content of *C. crambe* is above the mean of a range of sponges studied by McClintock (1987). McClintock (1987) reported that there was little correspondence between nutritional composition and feeding patterns of predators of Atlantic sponges. We have analysed the data of McClintock and found a positive relationship between energetic content and toxicity (Spearman $r = 0.611$, $n = 16$, $p < 0.02$) in contrast to what was found by Chanas and Pawlik (1995) on Caribbean sponges. Concerning the role of physical defenses, the sponge most effectively defended by chemicals in our case, *C. crambe*, also possesses spicules, while *D. avara* has a much less evident chemical deterrence, while lacking spicules and featuring a lower proportion of tough materials such as collagen. Lack of strong chemical deterrence (at least against grazers such as *P. lividus*) is not compensated for by structural defense in this case. The same result was obtained by Chanas and Pawlik (1995) in Caribbean sponges.

In conclusion, sessile stages of *Crambe crambe* (rhagons and adult forms) are well protected from predation by grazers (generalist consumers), whereas sessile stages of *D. avara* can be grazed by sea urchins. In contrast, larval stages are protected from predation in the latter species while they are unprotected in the former. Both sympatric sponges display a completely different strategy for protection from predation. These strategies are in agreement with other aspects of their respective life history such as reproduction investment and growth rates. A sponge with high turnover rates such as *D. avara* (current research) can be predated to a certain extent without strong detrimental effects, whereas a sponge with a slow growth such as *C. crambe* would disappear if sea-urchins fed on it. As in terrestrial plants (Coley et al., 1985) slow rates of growth would favour large investments in anti-grazing defenses. Clearly, our results should be

taken with caution because experimentation with a broader range of potential predators is required. Moreover, we were concerned only with the anti-predatory effects of chemical substances. However, these may also have other roles (antifouling -Becerro et al., 1994-, space competition -Turon et al., in press-) and their presence and function cannot be described only under the anti-predatory perspective. Schmitt et al. (1995) highlighted this fact, together with the constraints that the multi-purpose nature of chemical defensive mechanisms pose to co-evolution of chemically mediated predator-prey interactions. Even with these cautions, however, it seems clear that studies on anti-predation should take into account the different stages of the life-history of the organism studied, and use specific assay organisms at each relevant phase. When coupled with biological knowledge of the species, these studies may reveal patterns in defensive strategies and show their adaptive value.

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