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NATURAL VARIATION OF TOXICITY IN ENCRUSTING SPONGE *Crambe crambe* (SCHMIDT) IN RELATION TO SIZE AND ENVIRONMENT

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Abstract—The presence of intraspecific variation in toxicity and its relationship with biological or ecological factors were studied in the sponge *Crambe crambe*. Within-specimen (periphery and central part), between-size (< 1000 mm^2 in area, between 1000 and 10,000 mm^2 and $> 10{,}000$ mm^2) and between-habitat (well-illuminated and dark communities) variations in toxicity were evaluated by the Microtox bioassay. Quantitative differences were detected that were not attributable to within-specimen variation but to size and habitat effects. Habitat comparisons showed that sponges in the shaded habitat were significantly more toxic than those of the well-illuminated community. Sponges of the smaller size classes displayed significantly less toxicity than the medium-sized specimens. Results are interpreted under the optimal defense theory and their ecological implications are considered.

Key Words—Chemical ecology, natural toxicity, spatial variation, defensive strategy, encrusting sponges.

INTRODUCTION

The field of chemical ecology in marine environments is rapidly expanding, building on the field of the chemistry of marine natural products. During the last few decades, numerous new compounds have been isolated from marine

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organisms (e.g., see reviews by Faulkner, 1984, 1986, 1991), many of them with interesting pharmacological properties (Fautin, 1988; Hall and Strichartz, 1990). Recently, there has been increased interest in understanding whether such properties have a significant role in ecological processes. In the better-known field of terrestrial chemical ecology, laboratory and field investigations have led to better understanding of the role that chemistry plays in plant growth and germination (Rice, 1984; Carral et al., 1988), patterning of vegetation (Rice, 1984; Stowe, 1979), or plant–herbivore relationships (Rhoades and Cates, 1976; Harborne 1988), to cite some examples.

Defense mechanisms analogous to those reported for terrestrial environments may be expected in their marine counterparts, benthic communities. Similar pressures may determine the evolution of toxic compounds causing inhibition of growth in plants (Rice, 1984) and sessile organisms (Sammarco et al., 1983 Sullivan et al., 1983; Porter and Target, 1988) or inhibition of seed germination (Rice, 1984; Carral et al., 1988) and settlement inhibition (Davis et al., 1991). These processes may function as a strategy for space acquisition and maintenance, influencing spatial patterning. If toxicity has been selected as a defensive mechanism, it must have been as a consequence of coevolution with competitors (in a wide sense). When the nature of such competitors is environmentally determined, one can expect a variation of toxicity in relation to ecological conditions. These patterns are well documented in terrestrial environments and, together with experimental work, have provided the basis for modeling the production of chemical defenses. Variation in defense investment has been reported, for example, as a function of plant size, intraplant distribution, resource availability, growth rate, stress, and herbivore pressure (Feeny, 1976; Rhoades and Cates, 1976; McKey et al., 1978; Coley et al., 1985; Fagerström et al., 1987; Basey and Jenkins, 1993).

Although similar patterns might be expected in benthic communities, there is a lack of information on naturally occurring toxicity as compared with terrestrial ecosystems. Few studies have specifically addressed toxicity variation in benthic communities, although it has been detected in numerous species (e.g., bryozoans: Harvell, 1986; algae: Moon and Martin, 1985; Hay et al., 1988; Paul and Van Alstyne, 1988; gorgonians: Pawlik et al., 1987; Harvell and Fenical, 1989; soft corals: Coll et al., 1982; Harvell et al., 1993; and sponges: Thompson et al., 1985). To our knowledge, the works by Thompson et al. (1987), Yates and Peckol (1993), Harvell et al. (1993), and Maida et al. (1993) are the only studies where intraspecific ecological variation in chemical defenses was specifically addressed. Thompson et al. (1987) demonstrated that the variability in diterpene composition of the sponge *Rhopaloeides odorabile* is caused by the environmental conditions under which the sponge lives rather than to genetic differences. Yates and Peckol (1993) detected higher polyphenolic levels in *Fucus vesiculosus* in a community with low nitrogen availability as

compared with a nitrogen-rich site. Harvell et al. (1993) found quantitative and qualitative differences in the toxic compounds of the gorgonian coral *Briareum asbestinum* associated with site and depth. Higher levels of compounds were detected in corals in deeper waters, where levels of predation and productivity rates were lower (but see Paul and Van Alstyne, 1988, for contrasting results in a seaweed species). Maida et al. (1993) detected significant differences in the relative production of three toxic substances in the soft coral *Sinularia flexibilis* as a function of size and habitat. A greater production of a molecule involved in spatial competition was reported for specimens from a highly diverse and competitive site.

The present study examined the naturally occurring toxicity of the poecilosclerid encrusting sponge *Crambe crambe* (Schmidt). There were several reasons for choosing this species. First, *C. crambe* shows an unusually wide range of distribution. It is present in numerous western Mediterranean communities (from 0 to 60 m in depth), where it is one of the most abundant sponge species. Second, this species is known to possess an array of highly active metabolites (Berlinck et al., 1990, 1992; Jares-Erijman et al., 1991) with diverse ecological roles, such as antifouling, antipredatory, or as a space competition mechanism (Becerro et al., 1994a; Becerro, 1994). Third, most of the biological functions of sponges are accomplished at the cellular level. No circulatory or coordination system has been found, resulting in a lower degree of integration at the individual level with respect to other Metazoa. Moreover, sponges present an unusual organization; they are formed by the iteration of canal systems, which are labile modular units much more variable in space and time than the typical, more stable, units such as leaves or polyps. Taken together, these facts raise interesting questions as to whether the production of chemical defenses is coordinated in the whole sponge or whether there may be intraindividual variation.

Toxicity assessment can be performed through a fine-scale quantification of the molecules responsible for it using either HPLC (Harvell et al., 1993) or ^{1}H NMR (Maida et al., 1993). A second approach is to quantify toxicity in itself through a sensitive and precise bioassay. This approach was adopted in this study due to the high number of active compounds present in *Crambe crambe*. Besides, synergistic effects may be easily overlooked if separate quantification of molecules is performed. Furthermore, if a nontoxic compound can act synergistically with a toxic one, the former will not be considered at all unless its connection with the toxic agent is known. Berenbaum and Neal (1985), for example, documented in plants the increased effectiveness of a deterrent metabolite due to the action of a nondeterrent, cooccurring compound. The substances presumably responsible for the activity of *C. crambe* belong to two families of compounds: crambines and crambescidines, each with several molecules whose interactions are not known and whose isolation, even partial, requires a very laborious process (Berlinck et al., 1990). Therefore, we thought

it more advisable, in order to interpret ecological roles, to quantify the general toxicity per sponge unit weight, rather than trying to quantify one or several of these compounds.

The purpose of this study was to analyze the toxicity of *C. crambe* in relation to size and environment. Morphological changes, as well as variations in investment in structural components and reproduction as a function of size and habitat, have already been reported in this species (Becerro et al., 1994b; Uriz et al., 1995). The sponge seemed to respond to shaded habitats, which were space-limited, macrozoobenthos-dominated assemblages, by increasing directional growth and strengthening physical structures with increasing area. In contrast, specimens from well-illuminated, algae-dominated habitats showed an increase in organic matter, but not in allocation to structural components, with increasing size. Uriz et al. (1995) interpreted these results as a consequence of the space competitors present in the space-limited community. Accordingly, a higher investment in chemical defense may also be expected, and this is the hypothesis set forth in this study. Specifically, an attempt was made to answer the following questions: (1) is there intraspecimen variation in the toxicity of *Crambe crambe*? (2) is there intraspecific variation? and if so, (3) is this variation size dependent? (4) is this variation habitat associated? and (5) is there any relationship between the two factors?

METHODS AND MATERIALS

Sampling. All the specimens used in this study were collected in the locality of Blanes (NE of Spain, western Mediterranean), April 1992. The site was a 2 to 3-m-wide, 40-m-long gorge with north- and south-facing walls. Therefore, environmental conditions (temperature, food availability, underwater currents) were comparable on both walls, except for the amount of incident light. The north- and south-facing walls correspond to a shaded and a well-illuminated habitat, respectively. Communities on such walls showed remarkable differences in species composition: a space-limited community mainly dominated by encrusting organisms was found on the shaded wall, whereas the well-illuminated habitat was dominated by algae, and patches of free substratum were easily observed (see Becerro et al., 1994b, for a general description of these communities). Three size categories were established, according to results of Becerro et al. (1994b) in each of the two contrasting communities: (1) <1000 mm^2 in area, (2) 1000 to 10,000 mm^2, and (3) $>10{,}000$ mm^2. Ten specimens for each category of size and habitat were randomly selected. Samples were collected as follows: one small piece (≈ 1 cm^2) was taken from the central part of the sponge and three smaller pieces (adding up to approximately 1 cm^2) were picked up from the periphery in an attempt to reduce the expected heterogeneity

along the sponge border. Separate sampling in the center and periphery was not possible in the smallest size class, and intraindividual variability was assessed only in specimens from the intermediate and largest size classes. The samples were individually placed in plastic bags and taken to the laboratory for chemical extraction of the toxic compounds.

Extraction Procedure. A moderate number of active compounds are known in *Crambe crambe:* up to four crambines (plus several homologs) and four crambescidins have been described (Berlinck et al., 1990, 1992; Jares-Erijman et al., 1991) (Figures 1 and 2), and the list may not be complete yet. These compounds consist of a long, nonpolar chain with a polar head, which confer to them a mixture of lipophilic and hydrophilic properties, making them amenable to solution with solvents of different polarity. Preliminary studies were conducted to determine a method for easy extraction of these families of compounds. These studies are explained in full in Becerro (1994) and can be summarized as follows: (1) after trying all possible sequences with solvents covering a large range of polarities (hexane, dichloromethane, and water), we found that a single extraction with dichloromethane (DCM) takes up all toxic compounds, and no toxicity was detected if the tissues were extracted afterwards with any of the other solvents; (2) subfractioning the DCM extract with silica-gel columns in a gradient from petroleum–ether, petroleum ether–ethyl ether (decreasing ratios), ethyl ether, chloroform–methanol (8:2) to methanol showed that the toxicity was due to compounds in the most polar extreme of the range of metabolites solubilized by the DCM; (3) toxicity tests on the DCM fraction alone

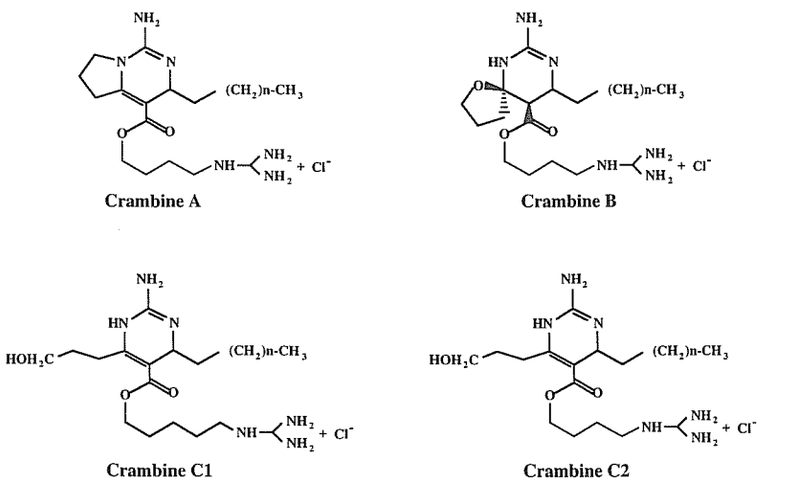
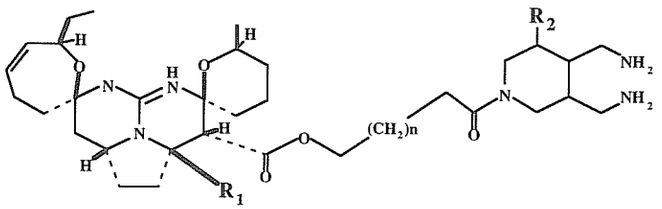


FIG. 1. Schematic representation of the crambines (from Berlinck et al. 1990, 1992).



Crambescidin 816	$R_1 = R_2 =$ OH, n = 13
Crambescidin 830	$R_1 = R_2 =$ OH, n = 14
Crambescidin 844	$R_1 = R_2 =$ OH, n = 15
Crambescidin 800	$R_1 =$ H, $R_2 =$ OH, n = 13

FIG. 2. Schematic representation of the crambescidines (from Jares-Erijman et al. 1991).

showed the same effect as that found after pooling the fractions obtained by successive extractions with hexane, DCM, and water. Thus, no synergistic effect with molecules not extracted by DCM alone was expected.

Once in the laboratory, each sample was carefully cleaned of any foreign body (such as polychaete tubes, barnacles, etc.) under a stereomicroscope and ground. Ten milligrams dry weight of sponge were then extracted three successive times (5, 15, and 30 min) with 5 ml of DCM (Becerro, 1994). The three fractions were pooled, the solvent evaporated under reduced pressure, and the residue homogenized in an ultrasonic bath in 20 ml of distilled water. This led to a concentration of 500 ppm relative to the initial sponge dry weight.

Toxicity Analyses. Since we wanted a fine quantification of the general toxicity of the sponge, we sought a biological test that permitted accurate toxicity readings. On the other hand, the test had to be ecologically significant if the results were to be interpreted in terms of the ecological role of chemical defenses. Several such roles are possible (Coll, 1992), and it is not feasible to devise quantitatively accurate tests for all potential ecological functions of the bioactive substances or for all potential competitors or predators. Instead, we engaged in a number of tests with ecological meaning (antilarval, antibacterial, cytotoxic, and antipredator with sympatric species) and tested their correlations with an easy, accurate test (Becerro, 1994; Becerro et al., 1995). As a result, we adopted a standardized procedure, the Microtox bioassay (Ribo and Kaiser, 1987; Kaiser and Ribo, 1988) as a precise method for measuring toxicity changes. This method is based on measurements of bioluminescence in living cell suspensions of the deep-sea bacterium *Photobacterium phosphoreum*. This proved to be the best test in terms of repeatability and precision and, although of little ecological meaning per se (it is hard to envisage how this particular bacteria

could affect *Crambe crambe* in nature), it was well correlated (Becerro, 1994; Becerro et al., 1995) with the other ecologically significant assays ($r = 0.98$ with the paper diffusion test with sympatric bacteria, and $r = 0.97$ with the sea urchin assay) and was used here as a precise tool for measuring variations in toxicity.

Freeze-dried bacteria were reconstituted at 4°C. In the basic procedure, Microtox requires four concentrations and one control per sample. The initial toxic concentration tested was 225 ppm (relative to sponge dry weight), and a dilution factor of two was used for the three successive concentrations (see Becerro et al., 1995, for a full description of this procedure). Experiments were run for 5 min at a temperature of 15°C. The light produced by the bacteria was recorded before and after the experiments in gamma units (GU):

$$GU = (R_t * I_0/I_t) - 1$$

where R_t is the correction factor (light differences in the control), I_0 is the light at time 0, I_t is the light at time t. Finally, the concentration at which GU is equal to 1 (50% of light reduction or estimated median effective concentration, EC_{50}) was calculated by fitting the data in logarithmic scale to a linear regression. The toxicity units (TU) used in this study measure quantitative changes in the samples and were calculated as $100/EC_{50}$.

Further information on the toxic behavior of the compounds can be obtained through the study of the slope of the regression function mentioned above. Two samples may have similar EC_{50} values but different slopes, indicating qualitative differences in the compounds responsible for the toxicity or changes in the relative amounts of these compounds. Consequently, the slope of the regression lines was used as a variable to measure qualitative changes in the samples. Differences in the slope indicate differences in the relationship between concentration of toxic compounds and toxicity, i.e., the higher the slope, the more effective the toxins.

Numerical Methods. Within-sponge differences in toxicity (center–periphery) were studied by paired-sample t-test analyses (Zar, 1984). These tests were performed for medium and large specimens (the only ones from which separate samples from the center and periphery were taken). The inclusion of the smallest size class for analysis of size and habitat effects rendered the data heteroscedastic (F_{max} test; Bakus, 1990) so that a rank transformation (Potvin and Roff, 1993) was applied prior to further parametric tests. Two-way analyses of variance (ANOVA) were performed, using size classes (three levels) and habitat (two levels) as factors. The former is clearly a fixed factor, and the second must also be considered fixed as the two communities studied were not selected at random, but specifically chosen because they were a priori expected to differ (Bennington and Thayne, 1994). We therefore restrict the conclusions to these two types of wall, and we do not mean to use the results as an estimate of the general variation

among all possible habitats. Whenever we speak of a habitat effect, therefore, we refer exclusively to the two habitats considered. Both variables, TU and slope, were analyzed by the same methods. The analyses were performed using the Systat 5.0 package.

Unplanned pairwise comparisons were performed following the Ryan's Q method (Einot and Gabriel, 1975; Day and Quinn, 1989), a stepwise procedure similar to the more familiar Student-Newman-Keuls (SNK) test, but which differs from it in using a lower α level at each step except the first one. Day and Quinn (1989) compared the performance of several multiple comparison methods using Monte Carlo simulation, and found that the Ryan's Q procedure produced the best approximation between the probability of type I errors and the nominal α level. The overall α for each set of multiple comparisons was set at 0.05.

RESULTS AND DISCUSSION

Results of the within-sponge TU variation are shown in Table 1. No toxicity differences were detected between the center and periphery of the sponges by the paired-sample t-tests, either considering all samples together or analyzing separately each combination of size and habitat. The two readings per sponge were therefore averaged to give a single value for further analyses. Table 2 summarizes the outcome of the two-way ANOVA of TU using size class and habitat as factors, whose values are graphically represented in Figure 3. Both main factors proved significant, and the interaction was not. A higher overall toxicity did exist in the sponges from the sciaphilous environment. Unplanned comparisons between size classes (across habitats) showed that the sole significant pairwise comparison (Ryan's Q, $\alpha = 0.05$) was the one between small and medium-sized individuals (the latter featuring higher toxicity, Figure 4).

TABLE 1. PAIRED-SAMPLE t-TEST ANALYSES OF TOXICITY (TU): INNER VS. PERIPHERY VALUES GROUPED BY HABITAT AND SIZE; OVERALL t TEST ANALYSIS ALSO SHOWN

Habitat	Size	Mean differences	SD differences	t	df	P
Sciaphilous	Medium	0.865	6.320	0.387	7	0.710
	Large	−2.998	6.457	−1.392	8	0.201
Photophilic	Medium	1.724	6.962	0.743	8	0.479
	Large	1.723	4.854	1.065	8	0.318
Overall t test		0.313	6.246	0.229	34	0.768

TABLE 2. RESULTS OF TWO-WAY ANOVA OF TOXICITY (TU) VALUES

Source	SS	df	MS	F	P
Habitat	1283.689	1	1283.689	5.888	0.018
Size	2936.717	2	1468.358	6.735	0.002
Habitat × size	1030.601	2	515.300	2.363	0.104
Error	11336.275	52	218.005		

The slope of the toxicity function also showed no within-sponge variation (Table 3) either analyzed by groups (habitat and size) or as a whole. Slope values for each habitat and size category are depicted in Figure 4. Two-way ANOVA of the slope values (Table 4) showed that neither the interaction term ($P = 0.604$) nor the main factors (habitat, $P = 0.691$; size, $P = 0.142$) had a significant effect.

The sponge *Crambe crambe* showed, therefore, a remarkable between-specimen variation in toxicity. This variation was related to size and habitat. Both factors significantly influenced the extent of toxicity found, although no qualitative differences were detected (as evaluated by the slope of the toxicity function) in the extracts. On the other hand, within-specimen (center–periphery) variation was not observed, in contrast to reports on other benthic organisms (Paul and Van Alstyne, 1988; Wylie and Paul, 1989; Harvell and Fenical,

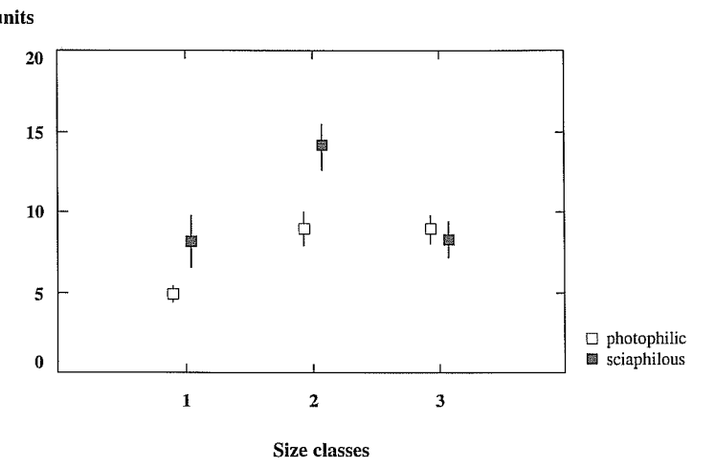


FIG. 3. Mean (±SE) values of the toxicity units (TU) according to habitat and size.

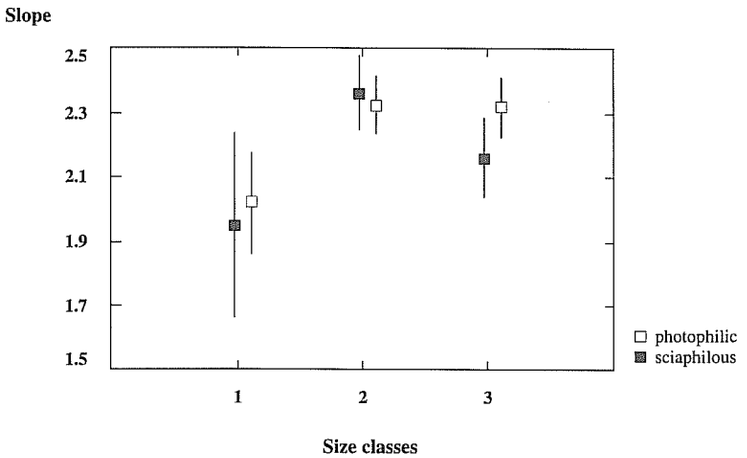


FIG. 4. Mean (±SE) values of the slope of the toxicity function according to habitat and size.

TABLE 3. PAIRED-SAMPLE *t* TEST ANALYSES OF SLOPE OF TOXICITY FUNCTION: INNER VS. PERIPHERY VALUES GROUPED BY HABITAT AND SIZE; OVERALL *t* TEST ANALYSIS ALSO SHOWN

Habitat	Size	Mean differences	SD differences	*T*	*df*	*P*
Sciaphilous	Medium	−0.041	0.733	−0.169	8	0.869
	Large	0.114	0.664	0.515	8	0.620
Photophilic	Medium	0.146	0.755	0.611	9	0.556
	Large	−0.130	0.442	−0.933	9	0.375
Overall *t*-test		0.021	0.642	0.204	37	0.839

TABLE 4. RESULTS OF TWO-WAY ANOVA OF SLOPE OF TOXICITY FUNCTION

Source	SS	*df*	MS	*F*	*P*
Habitat	45.369	1	45.369	0.159	0.691
Size	1151.225	2	575.612	2.022	0.142
Habitat × size	289.501	2	144.750	0.508	0.604
Error	14797.075	52	284.559		

1989). It was suggested in these studies that such differences were related to the chance of being attacked by herbivores, since chemical defenses were greater in those parts that lacked morphological defenses and/or were at a greatest risk or herbivory. No predators are known for *C. crambe*, but a comparable pattern against competitors for space could be expected. Although the sponges investigated did not present these expected variations and seemed to respond as a whole, unpublished results by the authors show that intraspecimen variation can be found in other seasons, corresponding to periods in which competition with neighbors is more intense.

Although changes in toxic composition have been reported for this species, and Berlinck et al. (1992) pointed out that the relative proportion of active substances in *C. crambe* changed in their samples, we did not find "qualitative" differences in toxic behavior in our study (measured as the slope of the toxicity function), indirectly suggesting that the composition of the toxic compounds remains fairly constant among levels of the factors investigated. Harvell et al. (1993) reported quantitative and qualitative differences in the chemical defenses of gorgonian coral. They provided evidence that qualitative composition was fixed and depended on geographic zone, while levels (quantity) of defenses were environmentally modulated, which is in good agreement with the results obtained in our study.

Interpretation of the quantitative differences found for toxicity (TU values) is based on correlational evidence, but under the assumption that the production of chemical defense compounds is metabolically costly, investment in defensive substances must be optimized with respect to the organisms' needs and to the other terms of the energy budget. This is the basis of the optimal defense theory (Rhoades, 1979; Coley et al., 1985; Fagerström et al. 1987; Skogsmyr and Fagerström, 1992), and from this perspective some explanations can be proposed.

Between-habitat differences must be interpreted as a function of the parameters changing from one wall to another. Water temperature, amount of food, and most abiotic parameters can be recognized as being comparable on the two walls. Light incidence, on the other hand, is clearly different between them. As no photosynthetic symbionts were observed in the many sponges studied by electron microscopy (authors' unpublished results), a direct effect of light on this species is not likely. The biotic interactions, on the other hand, were clearly different since diverse communities developed in both sites studied, as a consequence of the different amount of light received. The space is saturated in the shaded habitat, whereas patches of bar substratum are frequently produced in the well-illuminated habitat. Moreover, slow-growing, surface-dependent invertebrates or calcareous, encrusting algae are the dominant forms in the dark habitat, while the photophilic community was dominated by erect, soft seaweeds whose abundance changed markedly with season. The higher turnover of the

patches of bare substratum in this last habitat should favor investment in rapid growth when the conditions are favorable rather than in skeletal or chemical defenses (Jackson, 1977; Russ, 1982).

In contrast, the latter mechanisms should prevail in the shaded, space-saturated habitat. This hypothesis is supported by the different growth shape (Becerro et al., 1994b) and resource allocation (Uriz et al., 1995) of *C. crambe* in these two habitats. As a whole, therefore, we interpret the higher toxicity in the shaded wall as a result of differential strategies of space competition. The evolution of chemical defense strategies in marine organisms responds to highly diverse pressures (Bakus et al., 1986; Davis et al. 1989; Wahl, 1989), and more experimental work is needed to confirm the underlying process determining the pattern observed in this study. In particular, predation or fouling pressures should be considered, although they do not seem relevant in this case, as no predators or foulers have been observed in several years of study of this species in different localities and habitats.

Between-size differences can also be interpreted as a function of differential needs at each size stage. Encounters with space competitors are clearly a function of size, so a higher production of toxic substances with size was expected in principle. On the other hand, changes in toxic production with size may be tied more to internal, physiological parameters than to external factors. Although size and age are largely decoupled in modular, indeterminate-growing organisms (Sebens, 1987; Turon and Becerro, 1992), it is nevertheless expected that small sponges invest more in somatic growth and space acquisition, leaving fewer resources available for chemical defense.

The general pattern of variation found for toxicity values in Figure 3 points to an increase in toxicity from small to medium-sized specimens in both habitats, while the values remained constant (photophilic sponges) or decreased (sciaphilous specimens) from medium-sized to larger forms. In fact, if we restrict our analysis to the sciaphilous sponges and perform a Ryan's Q test among the three size classes, we find that the medium-sized specimens are significantly more toxic than the other two size classes. The lower toxicity displayed by the smallest and largest sponges can be due to the fact that they devote energy preferentially to growth and reproduction, respectively (an increased investment in reproduction with size has been found in this species; Uriz et al., 1995). Moreover, a more favorable area–perimeter relationship in large sponges may result in a lower energetic demand for defense interactions along the border (Adler and Harvell, 1993).

The values for the slope featured largely the same general pattern as the toxicity values (Figures 3 and 4). However, their large standard errors prevented significant differences from showing up in the analyses (possibly a sample size larger than 10 specimens per size and habitat would have been necessary). In fact, a Spearman correlation coefficient between the mean values of TU and slope per habitat and size showed that both were significantly correlated ($r_s = 0.942$, $N = 6$, $P < 0.001$).

In addition, *C. crambe* features highly effective toxins. The toxins are active at very small concentrations (Martin and Uriz, 1993; Becerro, 1994), and toxicity is rapidly enhanced by small increments in the toxin concentration, as indicated by the global values of the slope of the toxicity function (2.23 ± 0.05, mean $\pm$ SE). With such a slope, a 45% increase in toxin concentration would produce an approximately twofold toxicity increase. Both characteristics fit well with the hypothesis of a basal investment in toxic production and an ability for a precise regulation of the toxicity as a response to environmental pressures.

In conclusion, we did not find differences in toxicity between the center and the periphery of the sponges, although this study demonstrated that habitat and size exerted an influence in the levels of toxins encountered. The factors underlying the variation encountered in this study remain unclear, although the evidence suggests that space competition may be a key factor modulating toxin production. In this regard, Maida et al. (1993) also found higher levels of a molecule involved in spatial competition in specimens of the soft coral *Sinularia flexibilis* from a highly diverse and competitive site. The results of the present study also point to a chemically mediated interference competition, with higher toxicity in the sponges dwelling in the more space-saturated habitat. Whether this pattern of variation is followed by other chemically defended organisms is unknown. More research is needed to understand the processes modulating the production of chemical defenses and investment in biological parameters before an integrating perspective can be formulated.

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