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## Small-scale association measures in epibenthic communities as a clue for allelochemical interactions

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**Abstract** The small-scale associations in a rocky subtidal community in the northwestern Mediterranean were studied by a development of the continuous line transect method. This method allowed the overall measurement of non-randomness in interspecific contacts and the assignment of an association index to each species-pair, whose significance was tested by Monte Carlo procedures. At the same time, the continuous recording allowed the study of the weakening of the interactions with increasing distances. Our purpose was to uncover evidence for allelochemical mechanisms of space occupation and maintenance. A strong non-randomness was found in the interspecific associations. This was mostly due to the interactions of the pocillo-sclerid sponge *Crambe crambe* (Schmidt) with its neighbours, especially its negative associations with other sponge species. The strength of the relationships fell drastically over the first few centimeters from the contact borders of the different species. The results pointed strongly to an allelochemical mechanism. The extracts of this sponge featured high bioactivity in laboratory assays, and field experiments demonstrated that the sponge can inhibit the growth of species in the community studied. Standard sampling techniques would have overlooked the spatial structure present in the data. The study emphasizes the need for both contact data and distance data in order to identify the underlying pro-

cesses reliably. The line transect method provides both types of information easily and allows testing of models and identification of organisms likely to use chemical defenses in space competition. Its use as a preliminary step in studies of chemical ecology might help to detect presumptive allelochemical processes prior to experimental work on the potentially active species.

**Key words** Allelopathy · Spatial competition · Association · Epibenthic communities · Monte Carlo methods

### Introduction

The analysis of patterns of spatial relationships in any community is not only a descriptive exercise, but also a means to elucidate the factors and processes that determine these distributions. Here we aim to develop a technique for quantifying spatial interactions between sessile invertebrates on hard substrata. We address the question of how much we can tell about mechanisms and processes of interaction from distribution patterns, laying special emphasis on those that may result from allelochemical competition mechanisms.

Field methods for the study of structure in terrestrial and epibenthic assemblages fall into two main categories (Ludwig and Reynolds 1988): plot sampling, in which specimens are recorded within sampling units laid out over the surfaces studied, and point sampling (a family of plotless methods, so-called because they do not incorporate the use of areas), in which the structure is explained in terms of linear recordings of distances between points (Pielou 1959; Goodall 1965; Yarranton 1966; Loya 1978; Frontier 1983). Line transect methods (unidimensional) lie somewhere between plot (bidimensional) and point (zero-dimensional) methods, in that they allow recording of distances, as well as quantitative descriptions of the composition of the communities in real space (Loya 1972, 1978; Chiappone and Sullivan 1991). In the sense used here,

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line transect sampling involves recording in a continuous manner the species that lie beneath one line (the transect) placed across the community studied. It is important to note that this line is strictly unidimensional and has no breadth (Loya 1978).

As we were interested in uncovering evidence for chemically mediated interactions, our sampling design had to show, if present, any spatial structure likely to be due to the production of bioactive metabolites. A key hypothesis was that these substances act in a very limited spatial range, and thus small-scale structural patterns were of interest (Carral et al. 1988). It was assumed that short-range interactions are likely to be caused by factors deriving from the organisms (differential growth rates, food competition, physical or chemical defenses), while interactions at intermediate or large scales are more likely to be caused by environmental factors such as light, water movement and others (Stowe and Wade 1979). For this reason, we were interested in recording physical contacts as a sensitive means of detecting fine-scale interspecific associations (De Jong et al. 1980). Moreover, the continuous recording method allowed us to score species not only in contact, but at any distance apart along the transect line, thus offering the possibility of studying the course of the associations found with increasing distances.

The usual approach in studies dealing with interaction outcomes in epibenthic communities has been to emphasize the importance of overgrowth and growth stopping at contact edges of competing individuals or colonies, and such studies then focused on contact interactions (e.g. Buss 1980; Russ 1983). While these studies are useful in ascertaining competitive dominances determined by the above processes, they would overlook any competitive mechanisms acting at a distance. Our method allows determination of species which feature avoidance or non-contact interactions (i.e. are less in contact than expected) and of the changes in these interactions with distance. It is therefore, complementary to the former approach.

For the analysis of the results we modified a method first developed by Pielou (1967) and Stowe and Wade (1979), and already used in assessing allelopathy in terrestrial plant distributions (Stowe 1979; Carral et al. 1988). We introduced a Monte Carlo procedure (Manly 1991) for statistical inference on the results obtained. The simulation allowed us to determine the distribution of the statistics of interest in a null model of no spatial association between species and to compare the observed outcomes with this distribution.

It is difficult to distinguish, on the basis of distribution studies, between allelopathy and other mechanisms of spatial competition (Carral et al. 1988); complementary chemical knowledge and field or laboratory tests are usually called for. Some hypotheses, however, can be tested on the basis of spatial interactions alone. For instance, we expect that, if allelochemicals are involved in the interactions between two species, they will be in physical contact less often than

expected from their relative abundances, because chemical substances are likely to act at a certain (although presumably small) distance and thus produce a sort of gradient or inhibition ring surrounding the active species (Porter and Targett 1988; Sullivan et al. 1983), an effect that will be lost once the distance from the source of metabolites reaches a certain value. Other alternative mechanisms that could produce a similar "ring" effect (e.g. local food depletion, larval avoidance, grazing) should be considered before assigning an allelochemical nature to the interactions.

From the pattern of small-scale distribution, therefore, we can glean valuable information about underlying processes. We investigated this point using as a case study a sublittoral Mediterranean community where species known to possess strong chemical activities (as tested in laboratory assays, Uriz et al. 1991, 1992) are abundant.

## Materials and methods

### Field methods

This study was undertaken in Blanes, northeast Spain (Western Mediterranean; 41° 40.4' N, 2° 48.2' E). The site was a vertical wall facing north between 6 and 13 m in depth. This wall was covered by a visually homogenous sciaphilic assemblage mainly dominated by sponges (Becerro et al. 1994a). The sponge *Crambe crambe* (Schmidt) and a caespitous turf dominated by small algae with some bryozoans and hydrozoans were the most landscape-dominant forms.

A series of ten transects were laid horizontally on this wall in May 1993. Each transect consisted of a 5-m-long tape marked every centimeter. The initial position and the depth were chosen at random. Any specimen which lay beneath the line was recorded and its projected length on the tape was measured. This method allowed us to quantify the linear coverage of the different species and gave a continuous record of any contact along the transect. Samples of specimens that could not be identified underwater were collected for later identification. In order to allow for a comparison between direct and indirect methods, five such transects were recorded visually underwater, while the other five were photographed with close-up lenses (each picture covering c. 20 cm of transect) and analysed later in the laboratory.

### Test for non-randomness

The procedure for this test is summarized in Fig. 1. The recordings of species were converted into a single chain of letters (with breaks marking the separations between transects), each letter corresponding to one of the possible states (or species) found at every centimeter along the transects (the measures were previously rounded to the nearest centimeter; specimens less than 1 cm long were assigned 1 cm). As we were interested in the study of interspecific relationships, this chain was then collapsed, following Pielou (1967) and Stowe and Wade (1979), by replacing runs of the same species by single occurrences. This effectively ignores differences in size as well as intraspecific contacts. In this way we obtained a sequence in which there was a change in state from every step to the next (the size-structure of the original data is, however, used in the test for influence zones below). Pielou (1967) compared such a sequence to the chain which would be generated with the same states but in which the sequence of species was random. To this end, the transect was transformed into a transition or contact matrix in which the elements are the number of transitions

Test for particular associations

If the log-likelihood ratio test indicates a significant difference between the expected and observed values of the transition matrices, we can conclude that there is some association information present in our data. Clearly, we are not only interested in knowing that the sequence as a whole is non random, but we also seek a measure of the deviation from expectation for (a) particular species and (b) particular species-pairs. We first explain the method for measuring the association between species-pairs, and thence we derive the method for species. We used the following index to estimate the strength of association between species-pairs:

$$J_{i,j} = \ln \left( \frac{n_{i,j} + n_{j,i}}{m_{i,j} + m_{j,i}} \right)$$

where  $n_{i,j}$  and  $m_{i,j}$  were, respectively, the observed and expected number of transitions from species  $i$  to species  $j$ . They are simply the residuals  $\ln(\text{observed}) - \ln(\text{expected})$  summed for opposing elements of the matrix. They are 0 in the case of non-association, positive when species  $i$  and  $j$  are more in contact than expected, and negative if the reverse is true. This index is similar to the one proposed by Stowe and Wade (1979), but the latter was in multiplicative, rather than additive, form. We found that the multiplicative formula was slightly biased towards negative association values because small asymmetries (i.e. when  $n_{i,j} \neq n_{j,i}$ ) lower the product ( $n_{i,j} \times n_{j,i}$ ) even if the total number of transitions ( $n_{i,j} + n_{j,i}$ ) is the one expected.

From the observed and expected transition matrices, a triangular association matrix  $J_{i,j}$  can be computed (we added one transition to each cell before calculating the  $J$  indices to avoid the logarithm of zero indetermination). These  $J$ s provided an indication of the strength of the associations found.

Our analysis also diverges from that of Stowe and Wade (1979) in the way we assign a significance to  $J$  (that is to say, in determining when any of them is significantly different from 0). Those authors assumed a normal distribution of  $J$ , with a mean of 0 in the case of no association and a variance that can be derived analytically (although the calculations are not straightforward). This is a clear case in which distribution-free, computer-intensive methods may supersede and simplify other methods of analysis requiring strong assumptions and elaborate calculations (Manly 1991). We used a Monte Carlo approach to obtain the distribution of  $J$  under the model of non-randomness. To do this, we generated collapsed chains of equal length to the one observed by "sampling" the expected transition matrix at random. This was done by choosing a state at random among all possible states (considering their respective abundances), entering the transition matrix by the row corresponding to this state, and picking up a second element at random according to the expected proportions, then re-entering the matrix for the row corresponding to this second element and so on. For each chain generated we computed the corresponding triangular  $J$  matrix (Fig. 1).

The repetition of this process a large number of times (9999 plus the observed chain in our case) mimics the random variation of the  $J$  values in a population featuring random distribution of the states. We can then examine the position of the observed value in this distribution. This can be done by the straightforward method of ordering all the  $J$  values generated for a particular species-pair, leaving out, at the appropriate tail, a number of values (500 for a one-tailed 5% confidence interval on  $n = 10,000$ ) and checking whether the observed value is contained in the remainder of the generated distribution.

Similarly, we tested the overall contribution to non-randomness of each species by using the statistic:

$$S_i = \left( \sum_{j=1}^n |J_{i,j}| \right) / (n - 1)$$

which is the average of the absolute  $J$  values of all the association pairs of species  $i$  with other species. The significance of this statistic was tested by using the same ten thousand (9999 plus the

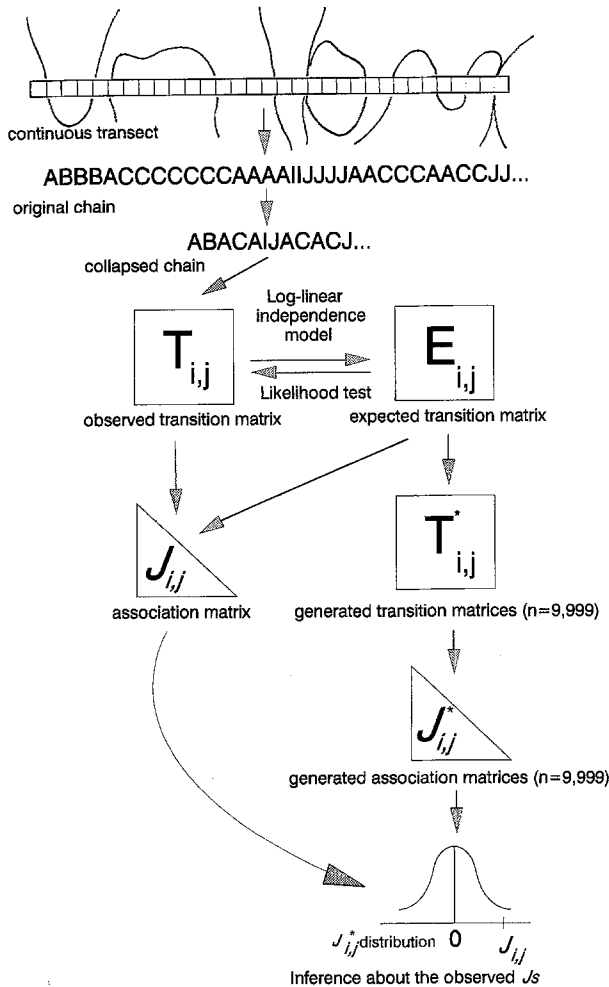


Fig. 1 Schematic outline of the procedures followed for obtaining and handling the data

(contacts) along the sequence from the state in the corresponding row to the state in the corresponding column. The diagonal was obviously 0 for a collapsed chain. All the transects were oriented in the same direction (i.e. from the shore to the sea) in the global chain, and it was "read" in one, arbitrarily chosen direction.

The transition matrix can then be compared by goodness-of-fit tests with the one expected under the random mingling model. There are several ways of computing this expected transition matrix. Pielou (1967) proposed an iterative method. De Jong et al. (1980) reported a maximization procedure for calculating the cell counts. Stowe and Wade (1979) used a log-linear model of quasi-independence (Knoke and Burke 1991) in which the diagonal elements were set to structural zeros. We tried all these methods using simulated chains with extreme conditions (for instance, by giving one of the states a percentage of appearance near the upper limit, 50%), and found that the log-linear method was the most robust to such extreme situations. Therefore, we estimated the expected transition matrix by fitting a log-linear model of quasi-independence to the observed transition matrix. The resulting matrix had the same composition as the original one, but featured independence of rows and columns, that is, independence of the second member of each transition from the first member, as required by the random mingling model. The fit of the observed and expected matrices was then evaluated using the likelihood-ratio statistic (Sokal and Rohlf 1981). The number of states in the chain was reduced to a manageable size by pooling the less frequent states (Pielou 1967).

observed one) samples generated from the expected transition matrix and computing their  $S_i$  values in order to determine their distribution under the non-random model.

Of course, when examining a set of statements such as the significance of the association values for every species or species-pair, we should be concerned with multiple comparison problems. We adopted a stepwise procedure for testing the significance of the associations: first, we tested the overall non-randomness (likelihood ratio statistic); if the overall test was significant, we tested the departure from randomness of particular species using the statistic  $S$  and correcting the error rate (overall level = 0.05) for the number of states being compared by a sequential Bonferroni procedure (Rice 1989); lastly, for the states that showed significant association information in the former analysis, we tested the  $J$  statistics of each state with the others, and corrected the error rate within state for the number of comparisons performed. In fact, it is the pattern of significant associations within the association table that is interesting (e.g. whether they are concentrated in one species), rather than the  $P$  value for any individual case.

#### Test for "influence" zones

The continuous recording method allowed for another possibility: the study of the species present, not in immediate contact, but at some distance from each contact border. At each transition in the uncollapsed chain, the element present at increasing distances of the contact was recorded as the second member of the transition. As before, in this way we obtained a transition matrix, fitted a log-linear model of quasi-independence and looked for goodness-of-fit between both matrices. Ideally, at some distance from the contact borders all the association information would be lost unless the chains showed some periodic properties. In addition, the evolution of the association indices between species and species-pairs at increasing distances could be tested using the same Monte Carlo procedure explained above.

One must, however, bear in mind two particularities of this approach with respect to the collapsed-chain method. First, the transition matrices obtained are increasingly asymmetrical, since originally each specimen was the second state of one contact at the proximal end and the first state of another at the distal end, but by this method large specimens continue to be the first member of only one transition (at their distal border), while they can "receive" several transitions from borders placed near them in the proximal part of the chain. Species with large mean sizes, therefore, would appear more frequently as the second members than as the first members of transitions. Second, what is generated is no longer a chain or sequence (when looking at some distance from a given border, we can "jump" over contacts but then we go back in the chain to find the following border), but simply a collection of transitions at given distances from the original borders which can be arranged in the form of a transition matrix. When resampling the expected transition matrices, therefore, we did not generate sequences as before, but simply picked up at random a number of transitions equal to the observed number.

#### Field tests

Field experiments were conducted on one of the species present in the study area, the sponge *Crambe crambe* (Schmidt), to determine whether its chemicals can play a role in space competition. Another of the encrusting sponges present, *Scopalina lophyropoda* Schmidt was known (authors, unpublished results) to possess a fairly high regeneration rate, which makes it amenable to short-term studies on growth inhibition. To this end, we selected at random 15 large (more than 2500 cm<sup>2</sup>) specimens of *S. lophyropoda* on the same wall as the association study, and scraped off a circular area (about 6.5 cm<sup>2</sup>) on each of them. Three treatments were then randomly assigned to the sponges. In five of them the holes were gently rubbed for 15 s with *C. crambe* collected in the vicinity; another five spec-

imens were used as a control for rubbing effects and were rubbed for 30 s with *S. lophyropoda*, while the remaining five sponges were left untouched. The duration of the rubbing was different to compensate for the fact that *S. lophyropoda* has less spicular content and, therefore, less abrasive properties. After 1 and 2 weeks the rubbing operation was repeated. The 15 holes were photographed at the beginning of the experiment, and after 1, 2 and 4 weeks. The images were then digitized and the area of the holes was measured.

## Results

The ten transects covered 5370 cm. A total of 932 transitions were recorded among the ten states recognized (48 species were identified, but the least abundant states were then pooled to give a final transition matrix of 10 × 10). These states are:

1. Complex: a seasonally fluctuating turf that covers the rock. It is composed of small caespitous algae (the most abundant at the time of sampling was the filamentous state of *Flabellia petiolata* (Turra)) mixed with some hydroids (*Sertularella* spp) and bryozoans (mainly *Nolella gigantea* (Busk)).
2. The sponge *Crambe crambe* (Schmidt).
3. The ascidian *Microcosmus sabatieri* Roule.
4. The sponge *Phorbas tenacior* (Topsent).
5. The bryozoan *Pentapora ottomulleriana* (Moll).
6. The sponge *Chondrosia reniformis* Nardo.
7. The sponge *Scopalina lophyropoda* Schmidt.
8. The crustose alga *Mesophyllum lichenoides* (Ellis) Lemoine.
9. Bare rock.
10. The Miscellaneous group, including all other species with very low frequency (the 35 species pooled in this category accounted for less than 15% of the length recorded).

The composition of the community, in terms of number of occurrences and of linear coverage, of the ten states considered, is shown in Fig. 2. As can be seen, the Complex and *C. crambe* are the dominant forms in both abundance and linear coverage. The Miscellaneous group is in third place, while the rest of the states featured much lower figures. The sizes (cm) of the different states (Fig. 3) showed that *S. lophyropoda* is the largest species found, followed by the patches of Complex. The remaining forms had a more or less similar patch size. As might be expected, the dispersion of the data was high, as indicated by the large standard errors. The average value of the patches of all states was 5.69 cm.

#### Association measurements

The five photographic and the five visual transects yielded ten transition matrices when considered separately or two transition matrices when pooled by sampling technique. Neither the transect variable (10 levels) nor the technique variable (2 levels) proved significant in log-linear analyses of these matrices (we compared

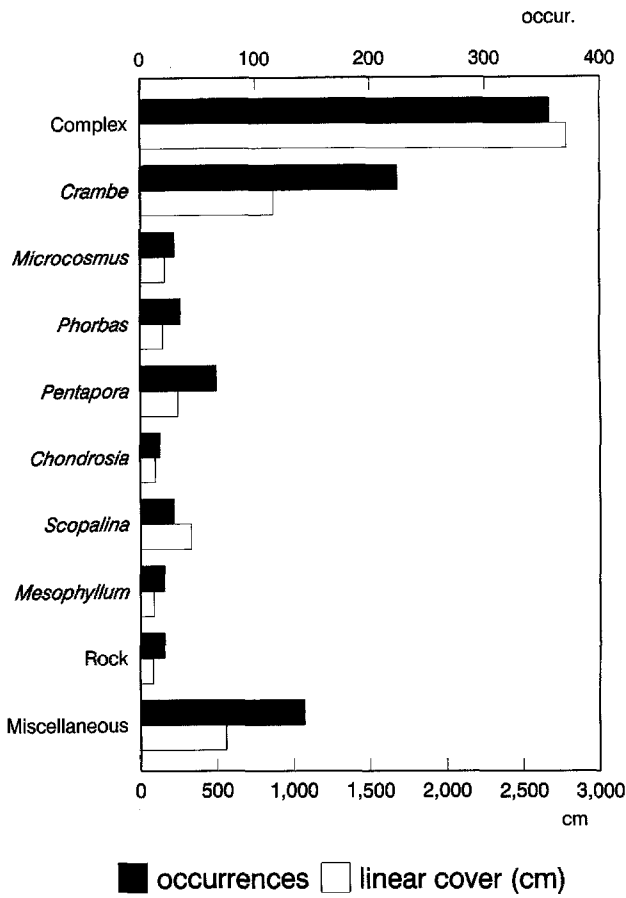


Fig. 2 Frequency (in number of occurrences) and coverage (in cm along the transects) of the ten states considered

Fig. 3 Mean values of the size (in linear dimension) of the ten states considered, as well as the global mean (All). Error bars are standard errors

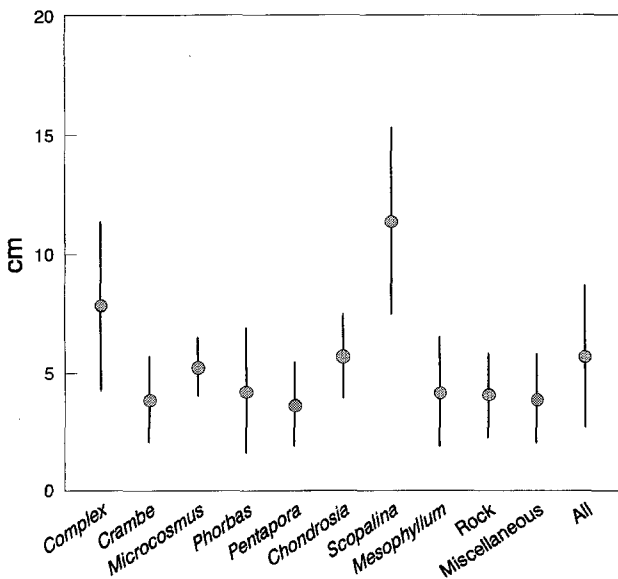


Table 1 Transitions observed (bold) and expected under the log-linear model of quasi-independence (italics) among the states considered in the ten transects studied

	Complex	Crambe	Microcosm.	Phorbas	Pentapora	Chondrosia	Scopalina	Mesophyl.	Rock	Miscel.
Complex	<b>0</b>	<b>164</b>	<b>13</b>	<b>20</b>	<b>38</b>	<b>12</b>	<b>18</b>	<b>8</b>	<b>2</b>	<b>76</b>
	<i>0</i>	<i>148.07</i>	<i>16.24</i>	<i>18.49</i>	<i>36.82</i>	<i>9.12</i>	<i>15.17</i>	<i>11.31</i>	<i>11.31</i>	<i>84.45</i>
Crambe	<b>150</b>	<b>0</b>	<b>6</b>	<b>3</b>	<b>12</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>11</b>	<b>37</b>
	<i>147.35</i>	<i>0</i>	<i>6.06</i>	<i>6.89</i>	<i>13.73</i>	<i>3.4</i>	<i>5.66</i>	<i>4.22</i>	<i>4.22</i>	<i>31.49</i>
Microcosm.	<b>13</b>	<b>5</b>	<b>0</b>	<b>0</b>	<b>2</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>6</b>
	<i>15.16</i>	<i>5.68</i>	<i>0</i>	<i>0.71</i>	<i>1.41</i>	<i>0.35</i>	<i>0.58</i>	<i>0.43</i>	<i>0.43</i>	<i>3.24</i>
Phorbas	<b>22</b>	<b>3</b>	<b>1</b>	<b>0</b>	<b>2</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>5</b>
	<i>18.47</i>	<i>6.92</i>	<i>0.76</i>	<i>0</i>	<i>1.72</i>	<i>0.43</i>	<i>0.71</i>	<i>0.53</i>	<i>0.53</i>	<i>3.95</i>
Pentapora	<b>46</b>	<b>8</b>	<b>3</b>	<b>5</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>3</b>
	<i>36.77</i>	<i>13.78</i>	<i>1.51</i>	<i>1.72</i>	<i>0</i>	<i>0.85</i>	<i>1.41</i>	<i>1.05</i>	<i>1.05</i>	<i>7.86</i>
Chondrosia	<b>7</b>	<b>0</b>	<b>1</b>	<b>1</b>	<b>2</b>	<b>0</b>	<b>2</b>	<b>1</b>	<b>0</b>	<b>2</b>
	<i>8.58</i>	<i>3.21</i>	<i>0.35</i>	<i>0.4</i>	<i>0.8</i>	<i>0</i>	<i>0.33</i>	<i>0.25</i>	<i>0.25</i>	<i>1.83</i>
Scopalina	<b>24</b>	<b>4</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>0</b>	<b>1</b>
	<i>15.68</i>	<i>5.87</i>	<i>0.64</i>	<i>0.73</i>	<i>1.46</i>	<i>0.36</i>	<i>0</i>	<i>0.45</i>	<i>0.45</i>	<i>3.35</i>
Mesophyl.	<b>8</b>	<b>3</b>	<b>1</b>	<b>0</b>	<b>3</b>	<b>0</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>3</b>
	<i>11.29</i>	<i>4.23</i>	<i>0.46</i>	<i>0.53</i>	<i>1.05</i>	<i>0.26</i>	<i>0.43</i>	<i>0</i>	<i>0.32</i>	<i>2.41</i>
Rock	<b>3</b>	<b>7</b>	<b>1</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>0</b>	<b>1</b>	<b>0</b>	<b>8</b>
	<i>11.29</i>	<i>4.23</i>	<i>0.46</i>	<i>0.53</i>	<i>1.05</i>	<i>0.26</i>	<i>0.43</i>	<i>0.32</i>	<i>0</i>	<i>2.41</i>
Miscel.	<b>77</b>	<b>30</b>	<b>4</b>	<b>5</b>	<b>6</b>	<b>2</b>	<b>5</b>	<b>7</b>	<b>7</b>	<b>0</b>
	<i>85.41</i>	<i>32</i>	<i>3.51</i>	<i>4</i>	<i>7.96</i>	<i>1.97</i>	<i>3.28</i>	<i>2.44</i>	<i>2.44</i>	<i>0</i>

**Table 2** Values of the association index ( $J$ ) for each species-pair calculated from the observed transition matrix. In parentheses is shown the proportion of the generated distribution above (for positive associations) and below (for negative associations) the observed value. For *Crambe crambe*, the values significant after sequential Bonferroni adjustment (at overall level of 0.1 or 0.05, see text) are *underlined*. The values of the  $S$  statistic are also listed, and those significant (after sequential Bonferroni adjustment at overall level of 0.05) are *underlined*

Complex	Crambe	Microcosmus	Phorbias	Pentapora	Chondrosia	Scopalina	Mesophyllum	Rock	Miscellaneous	S statistic
Complex	0.061 (0.150)	-0.176 (0.175)	0.122 (0.297)	0.129 (0.174)	0.064 (0.442)	0.292 (0.088)	-0.312 (0.094)	-1.257 (0.002)	-0.103 (0.114)	2.51
	<u>Crambe</u>	-0.055 (0.349)	-0.681 (0.014)	-0.294 (0.072)	-1.054 (0.002)	-0.659 (0.015)	-0.401 (0.099)	0.649 (0.006)	0.052 (0.326)	<u>3.906</u>
		Microcosmus	Phorbias							
			0.146 (0.236)	0.353 (0.198)	0.393 (0.161)	-0.476 (0.306)	0.325 (0.212)	0.037 (0.603)	0.316 (0.169)	2.277
				0.503 (0.065)	0.346 (0.196)	-0.542 (0.255)	-0.425 (0.363)	-0.425 (0.385)	0.187 (0.326)	3.378
			Pentapora		0.092 (0.490)	-0.890 (0.064)	0.381 (0.152)	-0.312 (0.143)	-0.482 (0.036)	3.435
				Chondrosia		0.397 (0.154)	0.178 (0.396)	-0.227 (0.604)	0.034 (0.490)	2.784
						Scopalina	0.329 (0.197)	-0.365 (0.444)	-0.076 (0.401)	4.025
							Mesophyllum	0.416 (0.145)	0.561 (0.062)	3.327
								Rock	0.909 (0.009)	4.597
									Miscellaneous	<u>2.720</u>

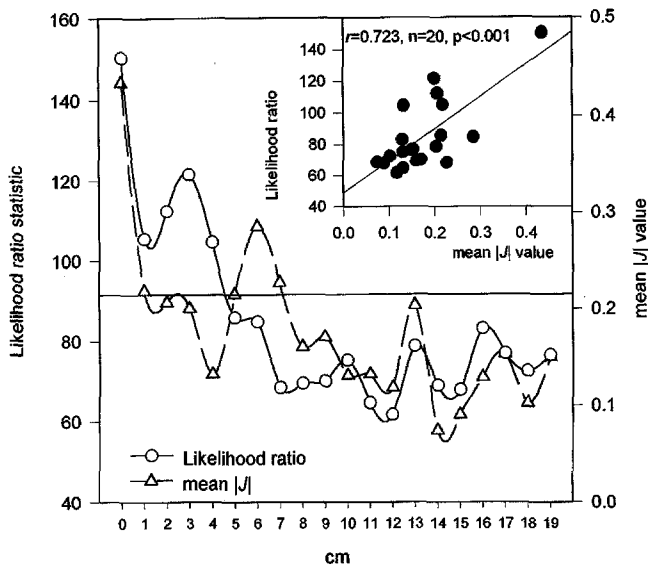
the fit of models with and without these variables and corresponding higher-level interactions, Sokal and Rohlf 1981). We then pooled them in a global transition matrix (Table 1), which was used for further analysis. One important point is that the matrix appeared to be symmetrical, which was confirmed by the symmetry test proposed in Knoke and Burke (1991). This indicated that there was no predominant direction of the competitive influences, at least along the horizontal dimension in the wall studied.

The transition matrix was tested with a model of quasi-independence, as described above, to obtain the expected transition matrix (Table 1) and the fit of the model was rejected (likelihood ratio  $\chi^2 = 150.42$ ,  $df = 71$ ,  $P < 0.001$ ), indicating the presence of an association structure in the community studied.

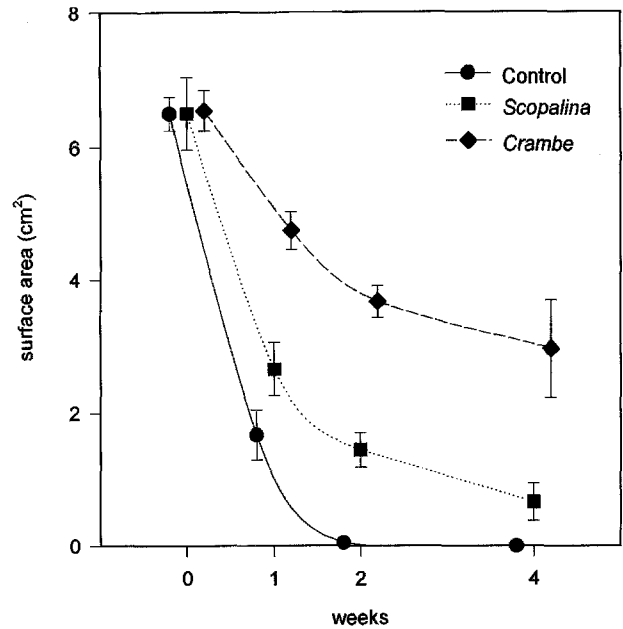
The association matrix  $J_{ij}$  was computed (Table 2). The values obtained for the overall association degree of each state (statistic  $S_j$ ) are also listed on Table 2. They were tested by the Monte Carlo procedure described and the significance level was adjusted using the Bonferroni sequential technique. *C. crambe* and Bare Rock featured significant values of this statistic.

Table 2 also lists the proportion of the distribution of the  $J$  values above (in the case of positive associations) or below (for negative associations) the observed value. This proportion can be considered as a one-tailed significance probability. If we use a Bonferroni correction for the nine  $P$  values of *C. crambe*, the associations with *C. reniformis* (negative) and Rock (positive) were significant at an overall alpha level of 0.05, and the negative associations with *P. tenacior* and *S. lophyropoda* were so at a 0.1 level. Manly (1995), in a study on species co-occurrences, considered outcomes under a error level of 0.1 as indicating possible interactions between species, and under a 0.05 level as indicating clear evidence for interactions. This same approach is adopted here. The study of the relationships of the other state that proved significant at the species level (Bare Rock) showed that this effect was due to the strong negative associations with *C. crambe* and with Complex (overall level  $< 0.05$ ).

We can also examine Table 2 on a comparisonwise basis, that is, looking for patterns of distribution of strong interactions. Setting aside two relationships of the Miscellaneous group with other states, which are inherently difficult to interpret because the former is a mixture of ecologically heterogeneous species, it can be seen that four out of five association values that reached a probability level below 0.05 involved the Poecilosclerid sponge *C. crambe*, the other one being the negative association between Complex and Rock. We can also see that the negative associations of *C. crambe* with *P. ottomulleriana* and *M. lichenoides* were also relatively strong ( $P = 0.07$  and  $0.09$ , respectively). It seems, therefore, that the relationships of the sponge *C. crambe* with its neighbours were responsible for most of the non-randomness detected in the interactions in the community studied.



**Fig. 4** Evolution of the likelihood-ratio  $\chi^2$  statistic computed when comparing the observed and expected transition matrices at increasing distances from the contact borders (in 1-cm intervals; the number in the *abscissa* indicates the beginning of the interval). The *horizontal line* marks the 0.05 probability level for this statistic and 71 *df*. The mean absolute  $|J|$  values of association between *Crambe crambe* and its neighbours at increasing distances are also shown. The correlation between the two variables is graphically displayed in the *inset*



**Fig. 5** Time course of the area of holes scrapped off in colonies of *Scopalina lophyropoda*. Control holes were left untouched, *Scopalina* treated holes were rubbed with *S. lophyropoda* at the end of one and two weeks. *Crambe* treated holes were rubbed with *C. crambe* at the same times

For comparative purposes, we can mimic the effect of measuring our community, not in a continuous manner, but sampling at equidistant points along the transects. This can be done by noting the state present in our original transects every *n* centimeters (the start was set at random within the first 10 cm of every transect). Thus, had we sampled the transects every 3 cm, we would have obtained a transition matrix that still revealed a significant departure from the independence model (likelihood ratio statistic = 133.98, *df* = 71, *P* < 0.001), but in which the only significant association found for *C. crambe* (after Bonferroni adjustment) was the negative one with *P. tenacior* (overall *P* = 0.1).

In order to explore the range at which associations were detectable, we tried the method of examining the states present at increasing distances from any contact border. The values of the likelihood ratio statistic provided a good indication of the course of the association information present if increasing distances are considered. The course of evolution of this statistic is shown in Fig. 4. There was a sharp decrease in the first centimeter (the log-likelihood ratio statistic changed from 152.25 to 105.44), and between 4 and 5 cm of the borders it fell below the 0.05 significance level, and remained so at least for the first 20 cm (we did not test any further), indicating that from a distance of approximately 5 cm from the borders the null hypothesis of independence between rows and columns (that is, of random mingling of states) could not be

rejected. In other words, the overall association structure in the original chain was lost when we examined the states present further than 5 cm from any contact border.

We can now test the relationship between the associations of *C. crambe*, the species that concentrates most of the significant interactions, and the global association information contained in the matrices at increasing distances. The average absolute  $|J|$  values of the associations of *C. crambe* with other states (statistic *S*) is also depicted in Fig. 4. This was taken to represent a measure of the strength of the associations of *C. crambe* with its neighbours at increasing distances. A clear positive relationship appears (Fig. 4, Pearson's correlation coefficient = 0.723, *n* = 20, *P* < 0.001) between this value and the global association measure represented by the value of the likelihood ratio statistic. In fact, the determination coefficient ( $r^2 = 0.523$ ) indicates that a good deal of the variation in the global measure of association can be explained by the course of the associations of a single species (*C. crambe*) out of 48 identified. Even if the point corresponding to the first centimeter is removed from the calculation, the correlation coefficient is still significant (at *P* = 0.05). It is clear from the graph that the associations of *C. crambe* sharply lost strength in the first few centimeters of distance. The test of the *S* statistic of *C. crambe* at increasing distances revealed that the overall association information of *C. crambe* was only significant in the first centimeter from its borders.

## Field tests

The course of the regeneration of the holes in *S. lophyropoda* under the different treatments is depicted in Fig. 5. After 2 weeks, three of the five control holes were already sealed off. We therefore compared the treatments after 1 week, since at that time all the holes were still in regeneration. ANOVA analysis (normality and homoscedasticity were assessed by Kolmogorov-Smirnov and Levene tests, respectively, and no transformation was necessary) showed a significant treatment effect ( $F$ -ratio = 19.5;  $df = 2, 12$ ;  $P < 0.001$ ). Post-hoc comparisons (Tukey test, Sokal and Rohlf 1981) showed that the holes treated with *C. crambe* had a significantly larger area that the other two treatments which, in turn, did not feature significant differences. The effect of rubbing with *C. crambe* was still noticeable at week 4, while a rubbing effect (as seen by the holes treated with *S. lophyropoda* still not sealed) was also apparent.

## Discussion

The method of continuous line transect sampling appeared to give satisfactory results both for descriptive purposes and for the study of interrelationships. The two sampling techniques used, visual and photographic, yielded essentially the same results. The former is preferable to ascertain the contacts with more precision, but the latter may prove useful when the time underwater is a limiting factor. The transect method has been applied to benthic assemblages for community structure characterization purposes (Loya 1972, 1978; Chiappone and Sullivan 1991). Few studies have exploited its potentiality for uncovering factors influencing pattern in benthos. Licuanan and Bakus (1992) used line-intercept data to study interactions in coral reef communities, finding non-random patterns in a high live-cover site. However, these authors only tested statistically the overall non-randomness.

A strong structure in the community was revealed by the highly significant departure of the observed transition matrix from that expected under the random mingling model. The testing of particular deviations of species and species-pairs from expectation revealed that most of the association information present was due to the interactions of *Crambe crambe*, which was involved in four out of the five strongest associations in the matrix (Miscellaneous group excluded). Essentially, what was found was a negative association of this sponge with the other three sponge species. The positive associations with the Rock and (to a lesser extent) with the Complex were interpreted as a consequence of the negative interactions with other states, rather than as intrinsic positive relationships. In fact, Complex and Rock acted as ubiquitous states occupying interstices between *C. crambe* and species negatively associated with it.

The strong negative association of Rock with Complex is also worth noting, as this was unexpected in principle. Their negative relationship may be an effect of slight changes in inclination in the wall, which caused some zones to be darker and devoid of the caespitous algal coverage. Where the light levels were slightly higher, the bare rock was nearly absent.

The method of examining transitions at increasing distances from the borders showed that the strength of the relationships fell drastically over the first few centimeters of distance. The overall association structure present was lost (at the chosen level of significance) between four and five cm from the borders. Further confirmation that *C. crambe* was a key species for the structure found was the significant positive relationship between the structure present at increasing distances from the borders (as measured by the global likelihood ratio value) and the mean absolute  $|J|$  values of the interactions between *C. crambe* and its neighbours (Fig. 4).

Summarizing, the application of the method in our Mediterranean community has revealed a pattern of association at small scale in which the interactions of a particular encrusting species, *C. crambe*, play the most significant role. These associations were lost at a few centimeter from the contact borders. What can be said about the mechanism(s) producing this pattern?

Non-contact outcomes cannot be due to overgrowth, one of the main space competition mechanisms. Larval avoidance behavior should be considered (Young and Chia 1981; Davis et al. 1991), but if larvae of some species tended to settle far from *C. crambe* (which would probably have to be interpreted as an allelochemical effect, Maida et al. 1995), this would not prevent contacts with the sponge after the initial growth, and the wall studied harboured a stable, well-developed community in which settlement occurred mainly after the period of our study (May), so newly settled organisms that could produce this effect were not abundant at the time of sampling. At larger scales, grazing has been documented to produce halo effects (Randall 1965; Witman 1987). The same can be found at smaller scales due to the action of small benthic grazers. In addition, some benthic organisms can retract in response to diverse cues (e.g. cnidarians, Sebens and De Riemer 1977; ascidians, Turon 1992), and this shrinking can produce a "ring" pattern. Encrusting sponges may also feature retractions (Ayling 1983). The possible shrinking and/or grazing effects should be kept in mind as potentially relevant. Against them, however, argues the fact that *C. crambe* featured only 14 contacts with empty space (Bare Rock), which is the state that would be expected in principle after a retraction or grazing event. Moreover, no predators are known for *C. crambe* (Becerro 1994).

Competitive mechanisms should therefore be considered as the most plausible explanation. Organisms on hard substrata can compete for light, food, or space (Buss 1986). Food competition may cause the pattern



observed, but it is unlikely that a thin encrusting form such as *C. crambe* would provoke any "shadow" effect to keep other filter-feeding organisms (mainly sponges) away, unless it has a much higher filtering rate. The other competitors are also in general encrusting, and so would be unlikely to exert any effect on *C. crambe* with the possible exception of *C. reniformis*, whose strong negative relationship could be influenced by a shadow effect of this moderately thick sponge. The same can be said of light competition, which, besides, seems less relevant as most of the interactions studied were between encrusting animal species. Moreover, no photosynthetic symbiont is harboured by *C. crambe* (Becerro et al. 1994b). Space competition appears therefore to be the most likely process underlying the observed distribution. A pattern of non-contact has been found in other sponges (Sullivan et al. 1983; Ayling 1983) known to possess toxins. Necrosis in nearby organisms due to allelopathic effects has been also demonstrated for soft corals (Coll and Sammarco 1983; Sammarco et al. 1983). On the other hand, sponges lack any known aggressive mechanism acting at a distance, such as the sweeping tentacles of some cnidarians or long vibraculatae in some bryozoans. Observational evidence from distribution studies therefore points to a chemically-mediated mechanism as the explanation for the structure found in the community studied.

To test this idea we resorted both to known evidence and to field experiments focusing specifically on *C. crambe*. It can be argued that the other species may also be chemically defended, and that what we are actually recording was their own bioactivity. However, any effect of the other states would be found in the interactions of these states, not only with *C. crambe*, but also with other species. It is informative in this sense that (apart from Rock for the reasons explained) *C. crambe* is the sole species showing an overall significant degree of association. At the species-pairs level, the significant interactions were concentrated in the second row (the one corresponding to *C. crambe*) of the association matrix.

*C. crambe* featured strong bioactivities in preliminary screenings against a variety of marine and terrestrial organisms (Uriz et al. 1991, 1992; Martin and Uriz 1992). It is known to possess an array of highly active metabolites (Berlinck et al. 1990, 1992; Jares-Erijman et al. 1991). The study of the chemical ecology of this species has been addressed from several points of view (Becerro et al. 1994a, b, 1995a; Turon et al. 1996; Uriz et al. 1996). The results obtained here from the small-scale distribution are fully consistent with the activity tests performed on extracts of *C. crambe* (Becerro 1994, Becerro et al. 1995b), which proved toxic against all marine microbial and invertebrate species tested, but which did not inhibit photosynthesis in the algae assayed, which agrees with its lack of effect on the algal turf. The toxic substances of *C. crambe* have been proved to act in preventing fouling by other organisms (Becerro et al. 1994a). We

lacked, however, direct evidence of growth inhibition in other species of the community studied. We selected for field experimentation on this point the sponge *S. lophyropoda* because it was negatively associated with *C. crambe* and because of its high regeneration rates. The rubbing assay showed that, even if rubbing itself has an unavoidable effect on the regeneration (possibly due to damage of the delicate growing edges), a strong effect due to *C. crambe* was found, attributable to the composition of this sponge. This experiment suggested that some compound from *C. crambe* prevented growth of one of its space competitors in the community studied. The long-lasting effect during several days and weeks is consistent with the structure of the toxic molecules of *C. crambe*, made up of a long, non-polar chain with a polar head which facilitates slow dissolution in water.

Recent research can explain how these molecules are released to the environment, a condition necessary for them to prevent physical contacts. The toxic substances in *C. crambe* are stored in the vacuoles of their large spherulous cells and a continuous release of such cells through the external layer of the sponge has been observed (Uriz et al. 1996), whereby the vacuoles' contents are shed to the surrounding water. This fact may account for the ability of *C. crambe* to exert an effect in the immediacy of its borders.

In conclusion, the method assayed permitted the identification of the relevant patterns of distribution. The method itself can easily be adapted for use in other kinds of environment where small-scale patterns are of interest. Although by themselves distributional studies do not demonstrate the nature of the underlying processes, careful examination of the results, especially when a pattern of non-contact is found, can give quite unambiguous clues to the allelochemical nature of the interactions. The need for small-scale recording seems clear: our simulated sampling of the community every 3 cm (a distance that would appear more than acceptable for most purposes) showed that most of the significant interactions of *C. crambe* with neighbouring states, that is, the ones most determinant of the structure found in the community, went undetected. A drawback of our study, however, is the fact that it is only a snapshot of a continuous process, and complementary studies over time seem advisable. Information about overgrowths and reversals would complete the picture of the space competition in the real community. Notwithstanding this remark, application of this method as a preliminary step in studies of chemical ecology may allow detection of suitable target species before engaging in more costly experimental work and thus save time and efforts in analyzing processes operating in the communities investigated.

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