Multi-scale patterns in co-occurrence of rocky inter-tidal gastropods along the west coast of India

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Over the last five decades, inter-tidal ecology has evolved from being a purely descriptive science to using quantitative and experimental approaches to understand community structure. However, this has sometimes come at the cost of understanding generality since many studies have focused on local spatial scales and patch dynamics. This study aimed to assess the generality of co-occurrence patterns at a large spatial scale, covering over 1000 km, by comparing empirical association patterns with random patterns generated by simulations. Species co-occurrence patterns are useful to examine the role of abiotic and biotic factors in community assembly. Our results show non-random patterns of species association at large spatial scales, mainly reflecting increasing community similarity with decreasing geographical distance, although random patterns of species association were found at the smallest spatial scales. Negative associations between species dominated the association pattern when all species pairs were considered, but most of the significant associations were positive. There was low congruence of association strengths of the same species-pairs in different clusters, indicating that associations are likely to be contingent on local conditions. Finally, association strength of con-familial species pairs was found to be higher than species belonging to different families, probably reflecting habitat filtering. This study emphasizes the role and importance of examining general patterns and of using a combination of experimental and observational studies to gain insights at multiple scales.

Understanding the structure of natural communities has long been a major goal of ecologists (Menge 1976). An important issue in community ecology relates to whether the species composition of a community is governed by non-random rules resulting in positive or negative associations among species. Such rules, if any, are likely to be influenced by physiological conditions (e.g. salinity, temperature, humidity, pH etc.), physical environment (e.g. wind, pressure, light, rain etc.), and biological interactions (e.g. competition, predation, disease and facilitation) (Bertness et al. 1999, Stachowicz 2001).

The concept of 'assembly rules' (Diamond 1975), explaining the composition of ecological communities, has been vigorously debated by ecologists (Gotelli and Graves 1996). One of the most influential of these rules, proposed by Diamond (1975), is that competitive exclusion among ecologically similar species results in mutually exclusive distributions. Although there has been substantial criticism of Diamond's assertion (Connor and Simberloff 1979), Gotelli and McCabe's (2002) meta-analysis of 96 published data sets showed empirical support for this assembly rule (but see Pitta et al. 2012 who tested about 270 data sets and showed that non-random associations were an exception and not a rule across taxa). Recently however, positive interactions among co-occurring species have also been emphasized, especially in stressful environments where one species or organism makes the local environment more favourable for another either directly or indirectly (Bertness and Leonard 1997, Stachowicz 2001).

Rocky inter-tidal shores have attracted ecological attention for many years because of the accessibility of the habitat, and observability and sessile nature of resident species (Connell 1961, Dayton 1971, Paine 1974, Garrity 1984, Connell and Irving 2008). Since this habitat encompasses strong gradients of environmental conditions within relatively short distances of the order of a few meters, patterns of response to environmental variables are likely to be relatively easy to detect (Newell 1976). High environmental turnover also results in a great diversity of plants and animals (Menge et al. 1986). Moreover, the species found in this habitat are small, abundant and easy to collect or manipulate, making these ecosystems suitable subjects for experiments (Underwood 2000).

Over the last five decades, inter-tidal ecology has evolved from a purely descriptive science to include experimentation to understand processes underlying distribution of intertidal species (Connell 1961, 1972, Dayton 1971, Paine 1974, 1994, Paine and Levin 1981, Schreider et al. 2003). For example, in one of the earliest studies, Menge (1976) examined competition for space between the barnacle *Balanus balanoides* and the mussel *Mytilus edulis* in the New England rocky inter-tidal habitat using a simple experimental design

with exclusion cages. However, Connell and Irving (2009) argue that the approach of focusing only on local spatial scales and patch dynamics has sometimes come at the cost of understanding generality. Witman and Roy (2009) review the progress of experimental marine macroecology and note that despite an increase in large-scale replicated manipulation experiments, such studies are still limited by the fact that the 'grain' (minimum resolvable area/area under manipulation) is small (usually > 2 m^2). Further, multiple variables, individually and in combination, act at different spatial–temporal scales making it difficult to control for all of them while conducting in situ experimental tests across a large spatial scale (Witman and Roy 2009). In general, there are few studies with hierarchical analysis of spatial patterns at large spatial scales (Fraschetti et al. 2005).

Community wide analyses of association at large scales are relatively recent, even in terrestrial systems, following the development of appropriate null models and computational power (Gotelli et al 2010). Recognizing the difficulty with testing association patterns using experiments at large scales, Gotelli et al. (2010) used alternative approaches such as null model analysis to study co-occurrence patterns of Danish avifauna at large spatial scales.

A hierarchical approach is necessary to understand the scales at which biotic and abiotic interactions occur. Documentation of patterns at different spatial scales aids in understanding processes underlying species occurrence and distribution (Underwood and Chapman 1996). Examining co-occurrence patterns helps in understanding community-wide patterns in species interactions. Unlike experiments, they are non-manipulative; however they are non-definitive tests of interactions and only indicative, since co-occurrence patterns may be a result of habitat segregation and other evolutionary factors (Gotelli and McCabe 2002).

Co-occurrence patterns can be studied for the community as a whole by using 'matrix-wide' indices (Gotelli and Entsminger 2001) or by examining associations between all the species-pairs in a given matrix called the 'natural-metric' (Sanderson 2000, used also by Sfenthourakis 2004, 2006, Pitta et al. 2012). Both metrics are able to recover deviating species pairs and provide similar but not identical results (Pitta et al. 2012). The pairwise approach helps to identify significant species associations in the overall community structure, while followers of the matrix-wide approach believe that by focusing only on species pairs, patterns produced by interactions among larger sets of species may be missed. There is little agreement over the approaches, but recent benchmark tests (Ulrich and Gotelli 2012) have suggested that using only community-wide metrics might be misleading and that pairwise approaches should be used instead (Gotelli and Ulrich 2010).

Given this background, we examined co-occurrence patterns in the gastropod community in rocky inter-tidal habitats along the west coast of India, using both community wide and pairwise approaches for comparative purposes. We assessed the generality of co-occurrence patterns by working at multiple spatial scales, covering over 1000 km by comparing empirical association patterns with random patterns generated by simulations. Competition is expected to be greater between more ecologically similar species (Diamond 1975). Therefore we also examined whether association strength was related to ecological similarity at the species pairwise level. Specifically, we 1) assessed whether the overall pattern of species association was random or non-random; 2) examined patterns of similarity in species composition between beaches to understand the influence of geographical distance; 3) determined the proportion of positive and negative interactions in the community and correlated pair-wise interactions in different clusters of beaches to test for congruence in patterns, and 4) examined whether the association strength of species was different between species pairs from same family and pairs with species from different families.

Methods

Study area

The study was carried out in the inter-tidal rocky shore ecosystem along the west coast of India, from February to April 2012. The study area extended from Arambol beach (15°41′N) in North Goa down to Kanyakumari (Cape Comorin; 08°04′N), covering almost 8° latitude (Fig. 1). The beaches comprised of low-lying, laterite and granite rock systems that support large numbers of gastropods and other associated species.

Three 'clusters' of beaches were identified to span the length of the coast, covering approximately 1100 km. Each of these clusters consisted of four predominantly rocky beaches, within a distance of 80–100 km of each other. The distance between clusters was approximately 300 km of essentially sandy beaches. The criteria for choosing the particular beach in each cluster were that it be rocky, wave-exposed and accessible. All sites were standardized by choosing those with the same orientation to sun and sea. The locations from north to south were: Arambol, Vagator, Anjuna and Palolem (in the Goa cluster); Ettikulam, Kannur, Kappad and Elathur (North Kerala cluster); Kovalam, Kurumpanai, Muttom and Kanyakumari (South Kerala and Tamil Nadu cluster, hereafter South Kerala; Fig. 1).

Larval dispersal of gastropods has been reported to extend up to approximately 10–150 km (Kinlan and Gaines 2003). Based on this, the three identified clusters were considered to be demographically independent populations, at least at short temporal scales.

Sampling

At each beach, one transect of 1–2 km was walked along the shore and approximately 20 vertical rocks (sampling sites) of 2–3 m height were sampled systematically. Distance between sampling sites ranged from minimum 10 m to a maximum of 50 m, depending on the size of the rocky inter-tidal area. Sites experiencing similar levels of wave-impact and exposure to sun were selected for sampling and all sites chosen were seaward facing, having the same orientation to the sun. All sites were sampled once during low tide. One quadrat of size 0.5 m^2 was laid per rock to establish gastropod species presence. Distinct zonation patterns are characteristic of intertidal fauna and in order to standardize the species found in the tidal zone, sampling was carried out in the mid intertidal zone (Dayton 1971). Thus the gastropod species
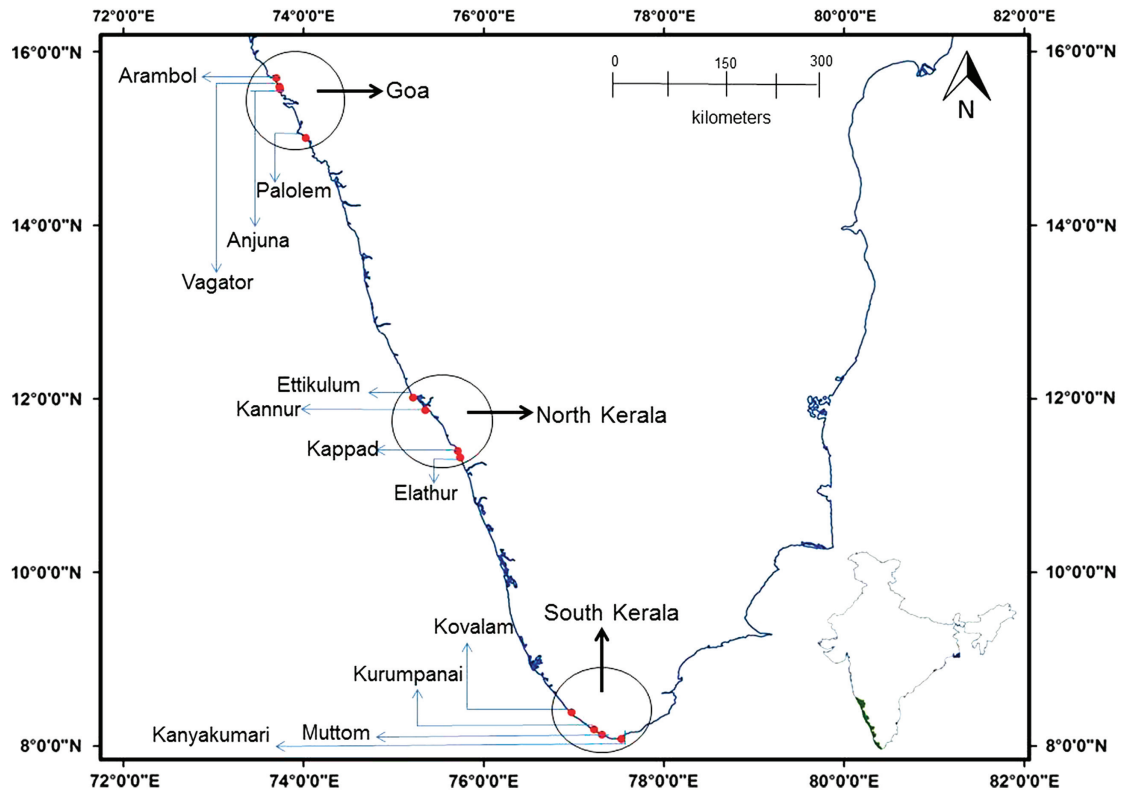
Figure 1. Rocky intertidal study sites along the west coast of India.

occupying this tidal regime were distinct in composition and do not experience extremities of exposure and submergence. The mid intertidal zone was calculated based on the average high tide and low tide levels in the sampled clusters provided by local tidal charts. The mid tide level at Goa was 1.3 m, North Kerala was 0.95 m and South Kerala was 0.65 m. The identification of organisms was carried out in situ whenever possible. Unidentified species were collected and preserved in 70% ethanol. Identification of collected specimens was carried out by following the keys of Sabelli (1979) and Dance (1992). Scaled photographs with rear and aperture view of all unidentified species were sent to the Natural History Museum, UK for confirmation.

Analysis

The occurrence of species at sites can be represented in a two-dimensional, $r \times c$, presence–absence matrix, where each row, r_i, represents a species, and each column, c_j, a site. Each cell in the matrix contains a '1' or a '0', indicating the presence or absence, respectively, of species i at site j. The row sum, Ri, represents the number of occurrences of each species, and the column sum, Cj, represents the number of species (species richness) at each site. Separate co-occurrence matrices were constructed for each beach, each cluster and one for the entire coast, with columns, in each case, representing sampling sites. Comparisons between matrices were used to arrive at generalizations in co-occurrence patterns.

Community wide co-occurrence patterns

Four common metrics are used to quantify community wide co-occurrence patterns: number of species combinations,

number of checkerboard distributions (i.e. the number of species pairs that shared no sites), Stone and Robert's (1990) 'checkerboard' index (C-score) and Schluter's (1984) variance ratio. Of these different metrics, the C-score is particularly useful because it is relatively insensitive to noise and not prone to type I errors (Gotelli 2000). Biologically, a community that has a significant community-wide C-score is consistent with the competition hypothesis (Gotelli and Entsminger 2001 but see Ulrich and Gotelli 2012). Therefore we used only C-score in our study. The C-score measures the average number of 'checkerboard units' among all possible pairs of species. The number of checkerboard units (CU) for each species pair is calculated as: $CU = (r_i - S)(r_j - S)$; where is S is the number of shared sites (sites containing both species) and r_i and r_j are the row totals for species i and j.

We used a null model approach implemented in EcoSim (Gotelli and Entsminger 2001) which can test for community patterns with non-experimental data. By randomizations, it creates 'pseudo-communities' (Pianka 1986) and then statistically compares the patterns in these randomized communities with those in the real data matrix. In order to determine whether patterns were random or not, 5000 simulations were carried out. For co-occurrence analyses, the most commonly used randomization is the 'fixed–fixed' algorithm (Gotelli 2000), in which species occurrences are randomized, but the row sums (= species incidences) and column sums (= species richness per site) of the observed matrix are preserved. The sequential swap algorithm (Gotelli 2000) was used to randomize species occurrences within a matrix. To determine the nature of pattern, we looked at the p-value and the standardized effect size, which is calculated

as: $(\mu_1 - \mu_2)/\sigma$, where μ_1 is the c-score of the observed matrix, μ_2 is the mean c-score of the simulated matrices and σ is the standard deviation of the simulated matrices. It scales the results in units of standard deviations, which allows for meaningful comparisons among different tests. A standardized effect size that is greater than 2 or less than -2 is considered to be statistically significant with a tail probability of less than 0.05.

Similarity

The species presence–absence matrix was used to calculate Jaccard's similarity index in species composition (Jaccard 1902, 1912, Hammer et al. 2007). Pairs of sampling sites were categorized as either belonging to the same or different beach. Randomized ANOVA (Gotelli and Entsminger 2001) was used to test for difference in species composition similarity between sites from same beach versus sites from different beaches. We used randomization version of parametric tests because pairs of sites were not independent. The analysis was repeated to compare similarities within and between clusters. Geographic distance between the beaches was calculated from GPS locations. Randomized Pearson's correlation implemented in EcoSim (Gotelli and Entsminger 2001) was carried out to examine the effect of geographical distance on similarity in species composition. Thousand simulations each were carried out for all randomization versions of parametric tests.

Pair-wise species associations and interactions

Pair-wise negative and positive association values were determined using the 'natural metric' (Sanderson 2000), implemented through the software CO-OC (Sfenthourakis et al. 2006). CO-OC identifies species-pairs that co-occur more or less often than expected by chance. It compares the actual co-occurrences of each species pair against the distribution of its co-occurrences in the simulated 'null' matrices obtained from EcoSim, and identifies the species pairs that show significant deviation, either at the lower end of the distribution (less co-occurrence than expected by chance, indicating a negative association between the two species) or at the upper end (more co-occurrence than expected by chance, meaning a positive association between the two species). While we were mainly interested in significant associations ($p < 0.05$), we also examined the relative proportions of all positive and negative associations to get an idea of the overall community pattern.

Pairwise association strength (deviation from the mean) $= (x - \mu)/\sigma$, where x is the observed number of co-occurrences between a species-pair, μ is the mean number of co-occurrences of the species-pair in the simulated matrices and σ is the standard deviation of co-occurrences of the species-pair in the simulated matrices. This was calculated for each species-pair.

Congruence of association strengths of the same species-pairs across clusters was examined using randomized Pearson's correlation. Species with fewer than three occurrences in a cluster were excluded from this analysis as association patterns for such species may not be robust. Other than these singleton and doubletons, all species-pairs were considered.

Association pattern among con-familial species

Competition theory predicts that competition is likely to be stronger, the more ecologically similar species are. Therefore one expects similar species to co-occur less than dissimilar species. To understand the influence of ecological similarity on association patterns, we compared association strengths of species within and across taxonomic families. Tests of competition are usually carried out at the congeneric level (Sfenthourakis et al. 2006); however this was not possible in our study because the dataset did not include an adequate number of genera with two or more species. All the species-pairs were divided into two sets – pairs of species in which both members belonged to the same family and species pairs in which members belonged to different families. Randomized ANOVA was used to examine the difference in average association strengths of the two groups.

All the above analyses were carried out at all spatial scales, i.e. at the scale of individual beaches, individual clusters and the whole coast. At the coastal scale, we also reran the analysis after pooling data from sites within a beach and treating beaches as replicates. As the results were similar to the analysis with plots as replicates, we focused on the latter to keep the analysis consistent across scales.

Results

A total of 27 species of gastropods were identified along the entire sampling area, with an average of 8.7 (± 2.7) species per beach and an average of 15 species (± 2.6) per cluster (Table 1a, b). Seven species were found to be unique to Goa, three to North Kerala and five to South Kerala; four species occurred in both Goa and North Kerala, one occurred in

Table 1. (a) Cluster-wise species distribution. (b) Beach-wise species distribution.

(a)

Species	North Kerala	Goa	South Kerala
Anachis cf fauroti	0	0	1
Anachis terpsichore	1	0	0
Cellana radiata	1	1	1
Clanculus scabrosus	1	0	0
Clypeomorus bifasciata	0	1	0
Clypidina notata	0	1	1
Conus sp.	0	0	1
Echinolittorina leucosticta	1	1	1
Echinolittorina malaccana	0	0	1
Echinolittorina vidua	0	1	0
Euchelus asper	1	1	0
Gyrineum natator	0	1	0
Littoraria undulata	1	1	1
Morula ceylonica	1	1	1
Nerita albicilla	1	1	1
Nerita chamaeleon	0	1	0
Planaxis sulcatus	1	1	0
Supplanaxis nigra	0	0	1
Thais blanfordi	1	1	0
Thais bufo	1	1	0
Thais konkanensis	0	1	0
Thais malayensis	0	1	0
Thais squamosa	1	0	0
Thais tissoti	1	0	1
Trochus stellatus	1	1	1
Turbo brunneus	0	0	1
Turbo intercostalis	0	1	0

(b)

Species	Elathur	Kappad	Ettikulum	Kannur	Anjuna	Vagator	Arambol	Palolem	Kanyakumari	Muttom	Kurumpanai	Kovalam
Anachis cf fauroti	0	0	0	0	0	0	0	0	1	0	0	0
Anachis terpsichore	0	0	1	0	0	0	0	0	0	0	0	0
Cellana radiata	1	1	1	1	1	1	1	1	1	1	1	1
Clanculus scabrosus	1	0	0	1	0	0	0	0	0	0	0	0
Clypeomorus bifasciata	0	0	0	0	1	1	0	0	0	0	0	0
Clypidina notata	0	0	0	0	1	1	1	1	1	1	1	1
Conus sp.	0	0	0	0	0	0	0	0	0	1	0	0
Echinolittorina leucosticta	1	1	1	1	1	1	1	1	1	1	1	1
Echinolittorina malaccana	0	0	0	0	0	0	0	1	0	1	0	1
Echinolittorina vidua	0	0	0	0	1	1	0	0	0	0	0	0
Euchelus asper	1	0	0	1	0	0	0	0	0	0	0	0
Gyrineum natator	0	0	0	0	0	1	1	1	0	1	1	1
Littoraria undulata	0	1	0	0	0	0	0	0	1	1	0	0
Morula ceylonica	1	0	0	1	1	1	1	1	1	1	0	0
Nerita albicilla	0	1	0	0	1	1	0	1	1	0	0	0
Nerita chamaeleon	0	0	0	0	1	0	0	0	0	0	0	1
Planaxis sulcatus	1	0	0	1	0	1	1	1	0	0	0	0
Supplanaxis nigra	0	0	0	0	1	0	0	0	0	1	1	0
Thais blanfordi	1	1	1	1	1	1	0	0	0	0	0	0
Thais bufo	1	0	0	0	0	1	1	0	0	0	0	0
Thais konkanensis	0	0	0	0	0	0	1	0	0	0	0	0
Thais malayensis	0	0	0	0	0	1	0	0	0	0	0	0
Thais squamosa	0	1	0	0	0	0	0	0	0	1	0	0
Thais tissoti	1	0	1	1	1	0	0	0	1	1	0	1
Trochus stellatus	1	0	1	1	1	1	0	0	1	1	0	0
Turbo brunneus	0	0	0	0	0	0	0	0	1	0	0	0
Turbo intercostalis	0	0	0	0	1	0	0	0	0	0	0	0

both North and South Kerala, one occurred in both South Kerala and Goa; while six occurred in all three clusters.

Community-wide results at the scale of coast, clusters and beaches

At the scale of the whole coast, observed co-occurrence was significantly less than expected by chance, (observed C-score = 102.7, expected C-score = 100.1, p = 0.009, standardized effect size = 2.6; Fig. 2a).

We also found statistically significant (p < 0.05) community-wide C-scores for the gastropod communities in all three clusters i.e. in Goa, North Kerala and South Kerala (Table 2, Fig. 2b–d). However, at the spatial scale of each beach, most locations showed a random pattern of species association (Table 3). Though not significant, the general trend for most beaches was also that observed C-scores were higher than expected by chance.

Similarity in species composition

Similarity in species composition was significantly larger within beaches (mean Jaccard's index = 0.30) than between beaches (mean Jaccard's index = 0.19; randomized ANOVA, p < 0.001) and within clusters (mean Jaccard's index = 0.48) than between clusters (mean Jaccard's index = 0.30; randomized ANOVA, p < 0.001). Geographical distance was negatively correlated to similarity in species composition (randomized r = −0.42, p < 0.001).

Pair-wise species associations

At the coastal scale, when all species-pairs were considered, 241 pairs were found to have a negative association pattern and 75 positive. However, when only significant associations among species pairs were considered, 21 pairs exhibited positive while only 8 showed negative associations. At the scale of clusters, a similar pattern was observed; when all species-pairs were considered, a higher number of pairs exhibited negative association (Fig. 3a), but the proportion of significant positive associations was much larger than significant negative associations for all three clusters of beaches (Fig. 3b). At the scale of individual beaches, no specific pattern could be observed (Table 4).

There was very low or no congruence in association strengths of species-pairs across clusters (Goa and North Kerala, r = −0.13, p = 0.65; North Kerala and South Kerala, r = 0.32, p = 0.13; Goa and South Kerala, r = 0.47, p = 0.05).

Association pattern among con-familial species

Results of randomized ANOVA at the coastal and cluster scale (all clusters) showed that con-familial species-pairs co-occur more than species from different families (Table 5). At the scale of individual beaches, no specific pattern was observed (Table 6).

Discussion

Community assembly has been of interest to ecologists for many decades (Diamond 1975) and has been studied in recent years using patterns of co-occurrence (Gotelli and McCabe 2002). However, few studies have been carried out at multiple scales in coastal and marine ecosystems (Fraschetti et al. 2005). Given this background, we aimed to study co-occurrence patterns in the gastropod community in rocky inter-tidal habitats along the west coast of India. Our
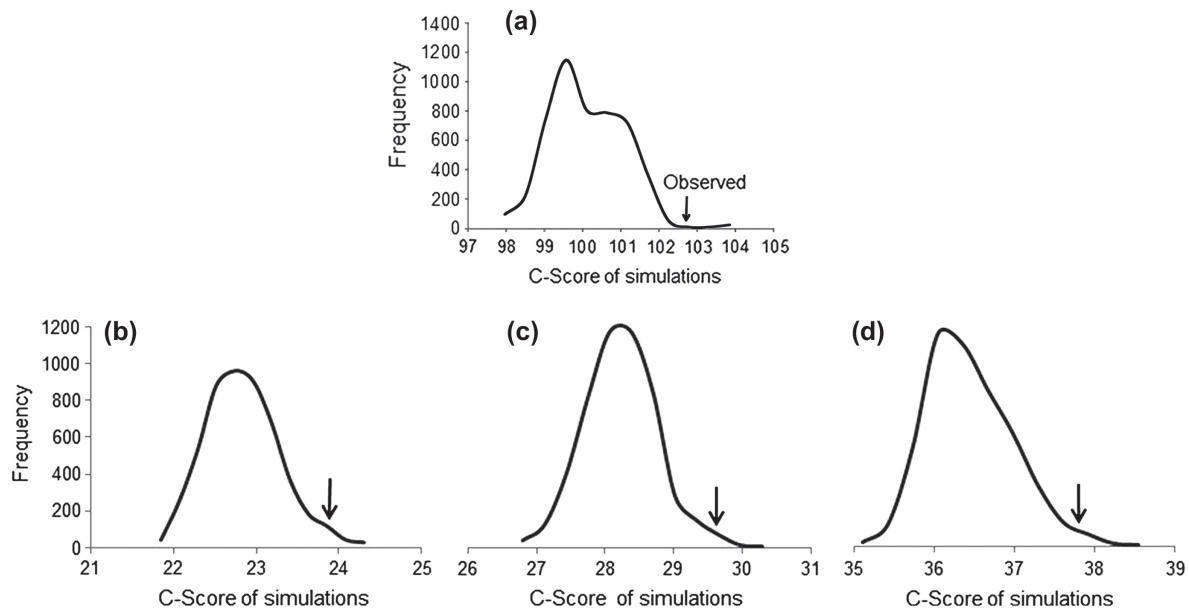

Figure 2. (a) Frequency distribution of c-score of null matrices at the coastal scale. The observed index shows the number of checkerboards in the real matrix, composed of the collected data. Since the number of observed checkerboards is larger than the number of checkerboards in the null (simulated) matrices, it implies that the communities are structured non-randomly; (b), (c), (d) Frequency distribution of c-score of null matrices at the cluster scale (Goa, North Kerala and South Kerala respectively). The arrows indicate observed number of checkerboards in the real matrices, composed of the collected data. Since the number of observed checkerboards is larger than the number of checkerboards in the null (simulated) matrices, it implies that the communities are structured non-randomly.

Table 2. Cluster-wise community-wide pattern of association comparing observed and expected checkerboard scores; all three clusters show a non-random co-occurrence pattern.

Locations	Observed C-score	Expected C-score	p-value (Obs ≥ Exp)	Std. effect size	Pattern
Goa	23.8	22.8	0.03	2.2	non-random
North Kerala	29.7	28.2	0.007	2.8	non-random
South Kerala	37.7	36.4	0.02	2.3	non-random

Table 3. Beach-wise community-wide pattern of association comparing observed and expected checkerboard scores; most beaches show a random co-occurrence pattern.

Locations	Observed C-score	Expected C-score	p-value (Obs ≥ Exp)	Std. effect size	Pattern
Anjuna	2.77	2.48	0.05	1.88	random
Arambol	6.93	6.74	0.21	0.82	random
Elathur	10.36	10.00	0.11	1.24	random
Ettikulum	3.93	2.96	0.02	2.97	non-random
Kannur	3.44	3.13	0.01	2.88	non-random
Kanyakumari	4.53	4.37	0.15	1.11	random
Kappad	2.13	2.32	0.86	−0.88	random
Kovalam	5.00	4.83	0.23	0.76	random
Kurumpanai	8.00	7.72	0.22	0.86	random
Muttom	4.13	4.20	0.64	−0.37	random
Palolem	1.73	1.74	0.58	−0.08	random
Vagator	4.58	4.50	0.25	0.69	random

results highlight the importance of studying general patterns and comparing them across different spatial scales.

Community-wide patterns

Biotic interactions are expected to occur at the smallest spatial scales (Dayton 1971, Connell 1972, Paine 1974, Menge 1976). However, this study shows that at the smallest spatial scales, across beaches, community structuring at different beaches is random (as also shown by Pitta et al. 2012). Further, community-wide measures of co-occurrence at the scale of 1000 km and 100 km (coastal and cluster level) show a non-random pattern of species distribution and association. Although these analyses show that the observed co-occurrences are less than expected by chance, they do not demonstrate that such non-random (structured) distribution is because of competitive biotic interactions between species, as per Diamond's assembly rules. If such a pattern is attributed

to competitive interactions, then the above pattern should repeat itself even at the smallest spatial scales, at which biotic interactions are most likely to take place. But at the scale of less than 10 km, i.e. at the level of individual beaches, this pattern does not emerge. Interactive processes such as predation and competition are known to have a strong influence at the patch level, but their effects seem to dilute at larger spatial scales where factors such as supply of propagules and demography seem to determine species organisation (Gaines et al. 2009). Moreover, as Sfenthourakis et al. (2006) have shown, such patterns might be due to evolutionary history
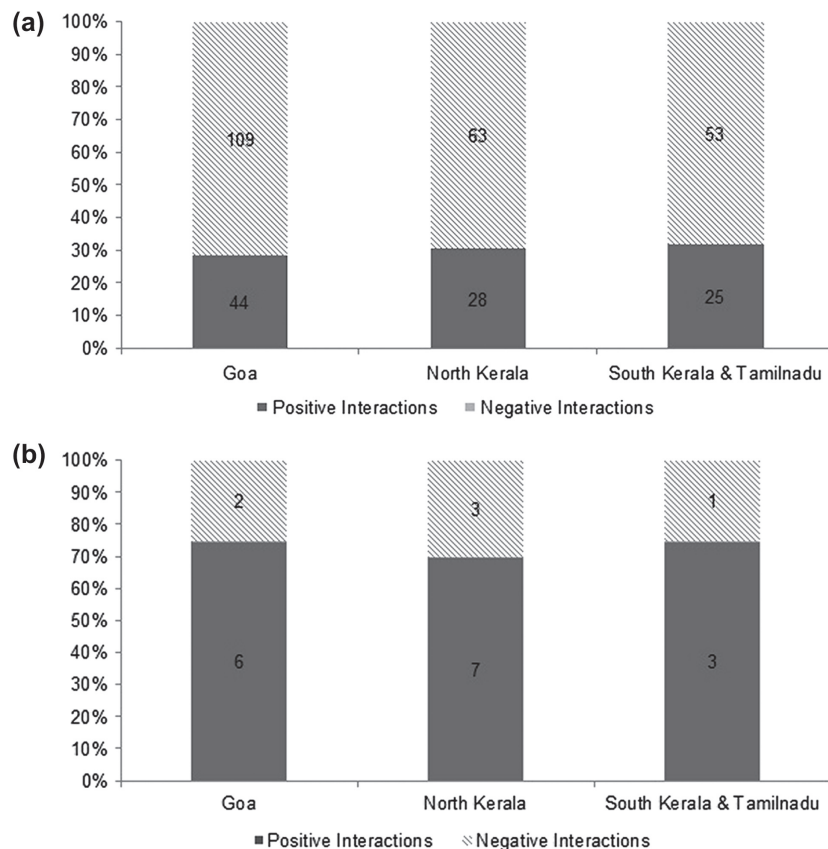

Figure 3. Percentage and number of interactions among species at cluster level scale for (a) all species-pairs and (b) for significant pairs (p < 0.05) respectively.

Table 4. Proportion of pair-wise species associations i.e. positive or negative interactions (represented as number of pairs and proportion) at less than 10 km scale.

Locations	Positive (p<0.05)	Negative (p<0.05)	Positive (all)	Negative (all)
Anjuna	1 (100%)	0	19 (24%)	59 (76.0%)
Arambol	0	0	14 (50%)	14 (50.0%)
Elathur	2 (66.66%)	1 (33.33 %)	15 (33.33%)	30 (66.7%)
Ettikulum	0	0	10 (66.67%)	5 (33.3%)
Kannur	2 (100%)	0	13 (36.11%)	23 (63.9%)
Kanyakumari	1 (100%)	0	16 (36%)	29 (64.0%)
Kappad	0	0	8 (53.33%)	7 (46.7%)
Kovalam	0	0	9 (42.86%)	12 (57.1%)
Kurumpanai	0	0	4 (40%)	6 (60.0%)
Muttom	0	0	15 (33.33%)	30 (66.7%)
Palolem	0	0	6 (40%)	9 (60.0%)
Vagator	1 (100%)	0	29 (31.87%)	62 (68.1%)

or ecological factors (exploitation of different or similar habitats).

Thus, the non-random pattern could just be a consequence of dissimilarity in species composition and reflect differences in the species pool. It is however possible that the non-random patterns do not appear at the smallest scale because of smaller sample sizes in comparison with the pooled samples at the cluster and coastal scale. The fact that C-scores were generally more than expected by chance (for nine out of twelve beaches), even if non-significant, suggests that this might be the case.

Supply of larvae is an important determinant of community structure in marine invertebrates (Underwood 2000). Populations can persist only if there is a constant supply of propagules, termed 'supply-side ecology' by Lewin (1986). Species can be involved in the structure of an assemblage only if it has arrived in the assemblage as larvae, and having arrived, managed to survive to a size large enough to escape predation (Underwood 2000). Thus, at the beach level, certain biotic interactions may not emerge, purely because some species have not reached there, and are not part of the community assemblage. Similarly, at larger scales, different beaches show signatures of different dispersal events, resulting in patterns of non-random co-occurrences and checkerboards. In other words, presence or absence of a species may simply be determined by larval supply and availability.

Species composition is likely to change over space, resulting in a distance decay of similarity, because each species has a limited distributional range, reflecting their niche-breadth and dispersal ability, and further, similarity in environmental variables decreases with distance along various environmental gradients (Cody 1985, Nekola and White 1999). In this study, we found that species composition appears to be more dissimilar with increasing

geographical distance between sites. Even though sampling was carried out on the same microhabitat, at the same tidal levels, species composition was more similar within beaches than between them. Even at the cluster level, species composition is more similar within clusters than between clusters. In a comparable study on rocky intertidal assemblages, Nakaoka et al. (2006) also found a significant negative relationship between similarity and distance for sites along the Japanese coast.

Pair-wise species associations

Apart from community-wide measures of co-occurrence, it is useful to identify particular pairs of species that contribute to the pattern of non-randomness (Gotelli and Ulrich 2010) as there may be certain significant pairs undetected in a matrix of mostly random associations (Sfenthourakis et al. 2006) many of which may not be biologically or statistically independent of one another (Gotelli and Ulrich 2010).

As above, the pattern of positive and negative associations between all the possible species pairs emerges only at the broader spatial scales of 100 and 1000 km. At both these scales, negative associations dominate the association pattern at the community level. However, the significant associations are mostly positive, i.e. species-pairs are co-occurring, suggesting that there are a few strong positive associations against a background of largely negative associations. This means that there are more negative associations at the community-wide level which are weaker, but the stronger associations are mostly positive. Sanderson (2000) and Sfenthourakis et al. (2004, 2006) note that an additional threshold of 5% should be used to check if the pairs are truly significant or due to chance alone, i.e. the significant associations should be more than 5% of the total species pairs. In this study, 15.8% of the associations were significant. It should be noted, however, that the species pool in this study is relatively small with less than 10 species on average per beach, and about 15 species per cluster. Positive association patterns may come about due to the presence of 'core species' at each site.

In our dataset, *Thais blanfordi* and *Cellana radiata* have the strongest negative association. This is probably explained by the fact that the predatory gastropods of the genus *Thais* are known predators of limpets and can determine their distribution (Menge 1973). Co-occurrence of herbivorous, pulmonate, gastropod species such as *Littoraria undulata* and *Echinolittorina leucosticta* among all clusters is probably explained by their similarity in habitat and resource requirements. Sfenthourakis et al. (2006) note that there is no apparent correlation between negative species associations and the C-score results, i.e. a community with significant C-score may have very few significant pairs with negative

Table 5. Result of randomized ANOVA test giving mean (μ), variance (σ²), p-value and standardized effect size of association (co-occurrence) between species belonging to the same family (confamilial) and different families (non-confamilial) at coastal and cluster scale.

	μ of confamilial	σ² of confamilial	μ of non-confamilial	σ² of non-confamilial	p-value	Std. effect size
Entire coast	0.62	2.96	0.04	1.80	0.03	2.72
Goa	0.28	0.88	0.04	1.89	0.53	−0.40
North Kerala	1.22	2.73	−0.08	1.45	0.01	5.42
South Kerala	1.80	2.08	−0.08	1.14	0.00	6.89

Table 6. Result of randomized ANOVA giving mean (μ), variance (σ²), p-value and standardized effect size of association (co-occurrence) between species belonging to same and different families at less than 10 km scale.

Locations	μ of confamilial	σ² of confamilial	μ of non-confamilial	σ² of non-confamilial	p-value	Std. effect size
Anjuna	−0.26	0.30	0.08	1.62	0.58	−0.42
Arambol	0.20	0.23	−0.05	1.49	0.77	−0.69
Elathur	1.51	1.79	−0.27	0.85	0.00	11.66
Ettikulum	0.33	0.00	0.07	1.98	1.00	−0.98
Kannur	0.58	2.78	−0.05	1.58	0.39	−0.16
Kanyakumari	−0.17	1.82	−0.02	1.02	0.79	−0.59
Kappad	0.83	0.00	−0.11	0.62	0.15	1.07
Kovalam	0.53	1.72	−0.09	1.30	0.42	−0.24
Kurumpanai	1.99	0.00	−0.27	1.31	0.09	2.44
Muttom	1.06	0.89	−0.11	0.68	0.01	3.87
Palolem	1.76	0.00	−0.14	0.86	0.07	2.61
Vagator	0.35	0.86	−0.05	1.00	0.30	0.01

associations and vice versa. Further, in their analysis of 272 published matrices of different taxa, Gotelli and Ulrich (2010) also find 40% of the most aggregated matrices to be that of poikilotherm groups (snails, ostracods and fish).

At the scale of less than 10 km, no clear pattern can be seen at the community level. This may be due to small sample sizes at the beach scale or that the patterns are actually random. It may also be possible that such interactions cannot be inferred from presence–absence matrices. Additionally, at small spatial scales at the rock (site) level, there may be micro-factors affecting distributions and associations between species. When all these factors are considered, patterns may get averaged or diluted, due to which no trends are visible. Again, at larger spatial scales, there may be other confounding variables, but our results show definitive patterns at this scale. Thus, at broader spatial scales, there may be certain patterns that may not be apparent by experimentation. On the other hand, clearly, experiments cannot be carried out at scales over hundreds of kilometers.

The low congruence of association strengths of same species pairs in different clusters suggests that it is difficult to generalize patterns of species associations based on sampling in one or a few locations. For example, the two species, *Morula ceylonica* and *Planaxis sulcatus* co-occur in North Kerala cluster, but 'compete' in the Goa cluster. This is probably explained by the differential predatory responses of *P. sulcatus*. For instance, *P. sulcatus* aggregates in crevices when crushed conspecifics are introduced (chemical cues indicating presence of shell-crushing predators) (McKillup and McKillup 1993). However, on introducing a drilling predator such as the genus *Morula*, the species were found to move to supra-tidal levels, beyond the reach of the predatory gastropods (McKillup and McKillup 1993). Results from patch level field and experimental studies, therefore, are limited in not being able to factor in spatial variations in a range of abiotic, ecological and behavioural determinants of species associations.

At scales greater than 100 km and 1000 km, the observed pattern shows that con-familial species co-occur more frequently than species-pairs belonging to different families. Though this is contrary to the belief that ecologically and evolutionarily similar species would exclude each other from the same communities through competition (Diamond 1975), it is likely that this pattern reflects the fact that similar species may utilize similar habitats and environmental conditions and therefore co-occur where such conditions are present (Webb et al. 2002, Cavender-Bares et al. 2009). The generality of competitive exclusion among con-generics was also not found in the analysis of 30 published presence–absence matrices by Sfenthourakis et al. (2006). They also suggest that factors other than taxonomic relatedness, like body size or functional relationships may play an important role in shaping co-occurrence patterns.

Conclusion

The study examined the co-occurrence patterns in the gastropod community in rocky inter-tidal habitats along the west coast of India. In summary, our results show a negative relationship between similarity and geographical distance, with non-random patterns of species association at large spatial scales. Negative associations between species pairs dominated the association pattern at the community level, but the few significant associations between species pairs were mostly positive, suggesting co-occurrence. Interestingly, there was low congruence of association strengths of species-pairs in different clusters, indicating that these associations were not consistent across regions. Finally, con-familial species co-occurred more frequently than species-pairs belonging to different families.

In recent years, the importance of scale in studying ecological and biogeographic processes has been particularly emphasized as patterns at different scales may be caused by different mechanisms (Rahbek et al. 2007, Evans et al. 2007, Nogués-Bravo et al. 2008). For example, large scale patterns may be largely influenced by historical geological and climatic processes, while small scale patterns may result from local environmental factors and biotic interactions. On one hand, since complexity tends to be the most at local scales, comparisons only highlight inconsistencies between different sites, and this variation can confound the search for generality. On the other hand, it is also possible that a multitude of local mechanisms result in similar large scale patterns, making it difficult to infer process from pattern (Oommen and Shanker 2005).

Clearly, the capacity and confidence in predicting local phenomena improves with experimental manipulation, but

it may not be possible to experiment at larger scales. Much of the work on inter-tidal fauna over the last few decades has been focused towards establishing associations through experiments, with the result that patterns have been ignored at larger scales. In this study, using the co-occurrence framework, we were able to uncover association patterns at large spatial scales that are likely to be indicative of species interactions. Further, community wide patterns such as proportions of positive and negative interactions in the community as a whole can only be derived from such an approach. The current study re-emphasizes the role and importance of examining general patterns and in comparing patterns across scales.

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