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Zonação em costões rochosos abrigados do Atlântico subtropical: Variação espacial das comunidades, efeitos do biofilme e da mortalidade diferencial de cracas

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“*Necesito del mar porque me enseña:*
No sé si aprendo musica o consciencia”

Pablo Neruda

Resumo

A zonação em costões rochosos é resultante da atuação de fatores físico-químicos e de interações biológicas sobre as assembleias bentônicas, caracterizando-se como um bom modelo para investigar os fatores que estruturam as comunidades biológicas. Neste trabalho, o objetivo foi avaliar processos envolvidos com um padrão de zonação particular em costões rochosos abrigados do sudeste brasileiro. O mesolitoral médio e inferior nesses ambientes constitui-se de uma zona majoritariamente formada por rocha nua, ou biofilme, apresentando apenas poucos indivíduos da craca *Tetraclita stalactifera* ou outros filtradores, enquanto o mesolitoral superior é dominado pela craca *Chthamalus bisinuatus*. Dessa forma, nós primeiro avaliamos a variação nas comunidades desses costões em três escalas espaciais (dentro de costão, entre costões e entre regiões), a fim de identificar a extensão do padrão (capítulo 1). Em seguida, elaboramos dois experimentos para testar as hipóteses de que 1) o padrão seria determinado pela ação do biofilme, o qual seria distinto nas duas zonas e influenciaria o recrutamento de cracas diferencialmente; 2) o padrão seria determinado por maior mortalidade de cracas na zona inferior, provavelmente causado por maior pressão de predação nessa zona (capítulo 2). Nossos resultados permitiram concluir que a principal fonte de variação nas comunidades estudadas geralmente ocorre em pequena escala espacial, indicando que os processos estruturadores dessas comunidades também devem ocorrer em pequena escala. O recrutamento de cracas durante o estudo foi excessivamente baixo e não correlacionado com as diferentes alturas do mesolitoral, enquanto o biofilme se mostrou mais abundante no mesolitoral inferior. Nesta porção do mesolitoral, as cracas apresentaram maior mortalidade do que na região superior, evidenciando forte efeito da mortalidade diferencial na estruturação das comunidades, apesar de terem sido amostrados poucos predadores nas áreas estudadas. Assim, este estudo permitiu colaborar com o conhecimento dos processos estruturadores em costões rochosos abrigados subtropicais, gerando um quadro de referência sobre o sistema estudado e subsidiando possíveis estudos sobre impactos ambientais.

Palavras-chave: Variação espacial, cracas, recrutamento, biofilme, mortalidade diferencial, costões rochosos abrigados subtropicais.

Abstract

The zonation of rocky shores results from the action of physical and chemical factors and of biological interactions within the benthic assemblages, being a good model to investigate factors that structure biological communities. In the present study, the objective was to evaluate the processes influencing a particular zonation pattern in sheltered rocky shores of Southeastern Brazil. The intermediate and lower midlittoral in these environments is a zone mainly formed by bare rock, or biofilm, presenting only few individuals of the barnacle *Tetraclita stalactifera* or other filtering-feeding species, whereas the upper midlittoral is dominated by the barnacle *Chthamalus bisinuatus*. In this way, we first evaluated the variation in these communities at three spatial scales (within shores, between shores and between regions), to identify the extension of the pattern (Chapter 1). Then, we carried out two experiments to test the assumptions that 1) the pattern would be determined by biofilm action, which would be distinct in the two zones and thus influence barnacle recruitment differentially; 2) the pattern would be determined by higher barnacle mortality in the lower zone, probably caused by higher predation pressure in this zone (Chapter 2). Our results allowed us to conclude that the main source of variation in the communities studied was usually at small spatial scales, meaning that the structuring processes in these communities would also occur at small scale. Barnacle recruitment during the study was excessively low and not correlated with different heights on the midlittoral, whereas the biofilm was more abundant in the lower midlittoral. In this midlittoral zone, barnacles showed higher mortality than in the upper midlittoral, evidencing strong effects of differential mortality structuring the community, although few predators were sampled in the studied areas. Thus, the present study contributes to our understanding of structuring processes in subtropical sheltered rocky shores, generating a reference framework on the system studied and subsidizing studies on environmental impacts.

Keywords: Spatial variation, barnacles, recruitment, biofilm, differential mortality, subtropical sheltered rocky shores.

Sumário

Introdução

Para determinar e mensurar o efeito dos impactos antrópicos nos ecossistemas, primeiro é necessário conhecer os mecanismos naturais de estruturação das respectivas comunidades (Underwood & Chapman 1998). A variação espacial nas comunidades reflete os padrões de distribuição (Boaventura et al. 2002), a diversidade e a coexistência de espécies (Ricklefs 2004, Cotteneie 2005), consistindo em um parâmetro relacionado com a estruturação destas comunidades. Assim, a avaliação das escalas de variação espacial constitui um requerimento crucial para entender os processos causais dessa variação, principalmente se aliado às abordagens experimentais (Benedetti-Cecchi et al. 2000).

A zonação em costões rochosos corresponde à variação espacial vertical da comunidade bentônica, a qual apresenta um padrão de distribuição de espécies em três zonas primárias, o supralitoral, o mesolitoral e o infralitoral [ver Sthephenson & Sthephenson (1949) e Lewis (1961, 1964) para classificações mais detalhadas]. Os organismos bentônicos pertencem a diversos grupos funcionais e taxonômicos (Koehl 2007) e a ocupação diferenciada desses organismos em zonas tem como principal fator determinante o potencial de dessecação, composto pela exposição ao sol e pela ação da temperatura e dos ventos (Bustamante et al. 1997, Bertness et al. 2006). Demais fatores atuam como moduladores, como a salinidade e a ação das ondas e das marés (Doty 1946, Underwood 1979, Ricciard & Bourget 1999, Westerbom et al 2002). Além destes, fatores biológicos como a competição (Connell 1961, Chapmam 1973, Branch 1984), a predação (Paine 1974, 1981, Willians et al. 2000, Navarrete & Castilha 2003) e a facilitação (Harlley 2006, Bulleri 2009, Thomsen et al. 2010), também são responsáveis pelos padrões. Usualmente, os limites superiores de distribuição das espécies são limitados pela ação dos fatores físicos, enquanto as interações biológicas são mais influentes na determinação dos limites inferiores (Connell 1961). Para os organismos bentônicos que possuem desenvolvimento indireto, incluindo uma fase dispersora ou larval, o recrutamento também pode influenciar os padrões de zonação (Sutherland 1990, Ank et al. 2009), adicionalmente às interações biológicas já citadas, incluindo uma combinação de processos pré e pós-assentamento. É possível haver forte efeito do recrutamento na estruturação da comunidade, uma vez que deve haver suprimento de larvas suficiente e local propício ao assentamento dessas para o estabelecimento da população adulta (Gaines et al. 1985, Jenkins et al. 2000, Skinner & Coutinho 2002).

As cracas (Cirripedia; Thoracica) são organismos bentônicos com desenvolvimento indireto e que podem ser formadores de faixas de dominância na comunidade do mesolitoral (Boaventura et al. 2002). O recrutamento das cracas é passível à influência tanto de fatores na fase larval quanto no assentamento (Pineda et al. 2002). As larvas são dispersas por correntes de água e podem ser mais ou menos seletivas quanto ao local de fixação (Crisp 1955), dependendo da qualidade nutricional da fase naupliar, o que interfere no tempo disponível para seleção do local de fixação (Thiyagarajan et al. 2002). Além disso, a determinação da posição vertical dos adultos na rocha pode ser influenciada pelo posicionamento ou agregação das cipris na coluna d'água (Grosberg 1982). Com relação à qualidade do habitat, a adequação do local para o assentamento depende das características do substrato, tais como o tipo de rocha (Tanaka & Duarte 1998), sua textura e micro heterogeneidade (Chabot & Bourget 1988, LeTourneux & Bourget 1988, Hills & Thomason 1996), a presença de substâncias químicas (Roberts et al. 1991, Pawlik 1992), bem como a presença prévia de outros organismos no local.

Há interações que podem gerar uma influência positiva na colonização dos cirripédios, como a causada pela presença de coespecíficos (Knight-Jones & Stephenson 1950, Crisp & Meadows 1962, Crisp & Meadows 1963). Neste caso, as larvas respondem a indutores químicos derivados dos indivíduos de sua espécie, tanto dos adultos, quanto das próprias larvas, o que é um estímulo para o padrão de agregação encontrado no assentamento desse grupo (Hadfield & Paul 2001, Berntsson et al., 2004).

A presença de competidores é um dos principais fatores com influência negativa na colonização das cracas (ver Underwood 2000 para revisão). Essa relação pode impedir o assentamento por influência física, como quando a prévia cobertura de algas na rocha limita a fixação da cypris (Dayton 1971). Mas também há substâncias capazes de inibir o assentamento, as quais podem ser encontrados na superfície de corais (Targett et al. 1983) e de macroalgas (Bazes et al 2009), por exemplo. A estratégia ecológica dos organismos marinhos que têm capacidade de amenizar a pressão de incrustação usualmente é alcançar proteção contra os epibiontes em longo prazo (Ralston & Sawin 2009). Assim, a competição pode atuar diretamente na zonação. A competição também pode influenciar a zonação após o assentamento, através da mortalidade de organismos por sufocamento (Denley & Underwood 1979). Um clássico estudo (Connell 1961) mostrou que a craca *Balanus balanoides* permanece na faixa mais inferior do mesolitoral enquanto a craca *Chthamalus stellatus* ocupa a porção mais superior em decorrência da maior habilidade competitiva da primeira, causando o sufocamento de *C. stellatus* na zona inferior.

Os predadores atuam negativamente sobre as populações de cracas, aumentando a mortalidade tanto de recrutas quanto de adultos, o que gera efeitos diretos na distribuição e abundância desses filtradores (Paine 1981). A predação de cracas pode ocorrer pela ação de diversos organismos, com destaque para gastrópodes dos gêneros *Stramonita*, *Leucozonia* e *Morula* em costões rochosos subtropicais (Magalhães 2000). Os predadores podem influenciar os padrões de zonação através da eliminação de indivíduos nas zonas mais baixas, onde os primeiros são mais abundantes (Connell 1970). A predação de cracas ainda pode ocorrer pela atuação dos próprios coespecíficos, ao ingerirem nauplios, o que influencia nos padrões de recrutamento (Navarrete & Wieters 2000).

O complexo de bactérias, diatomáceas, protozoários, fungos e compostos orgânicos dissolvidos do biofilme, presente na superfície de costões rochosos, também influencia o recrutamento de cracas. Na realidade, o biofilme foi tido como requisito para a incrustação de macroorganismos, sendo considerado uma fase pioneira na sucessão ecológica de substratos consolidados (Wahl 1989). No entanto, alguns estudos com diferentes invertebrados (Crisp & Ryland 1960, Wieczorek & Todd 1997), incluindo as cracas (Mary et al. 1993), sugerem que o complexo de microorganismos pode causar redução do assentamento desses grupos. As diferentes respostas no assentamento podem ser devidas a diferenças estruturais do biofilme (Wieczorek & Todd 1998). Essa variação das características do biofilme pode ser decorrente de diferenças nos fatores abióticos, como temperatura (Nasrolahi et al. 2012), salinidade (Lau et al. 2005), suprimento de nutrientes (Firstater et al 2012), características do substrato (Faimali et al. 2004), grau de imersão e ação das ondas (Thompson et al. 2005).

A abundância e composição do biofilme correspondem a fatores variáveis com influência no recrutamento, a exemplo do assentamento de *Balanus amphitrite*, que variou com a densidade de bactérias, a qual aumenta com o tempo de imersão e com a idade do biofilme (Oliver et al 2000). Henschel e Cook (1990) encontraram que o assentamento de cracas poderia ocorrer em biofilmes com apenas um dia de desenvolvimento e Qian et al. (2003) relataram que as cipris preferiam biofilmes com idade moderada (entre seis e nove dias), provavelmente por haver efeito indutivo inicial das bactérias, então neutralizado por demais componentes microbianos desenvolvidos a posteriori, como as diatomáceas e cianobactérias. É conhecido que a matriz de substâncias extrapoliméricas do biofilme pode ser uma fonte de sinais para as cracas (Wieczorek et al. 1996) e que essa pode concentrar substâncias do ambiente através da absorção (Thiyagarajan 2010); se as substâncias absorvidas forem proteínas dos coespecíficos, o biofilme pode ter efeito positivo no assentamento (Henschel & Cook 1990). Assim, sinais do biofilme podem ser significantes na determinação da variação espacial no recrutamento (Hung et al. 2007). Portanto, características do biofilme podem influenciar a zonação dos costões rochosos: Thompson et al. (1998) encontraram maior assentamento de cracas em biofilmes oriundos da região entremarés em relação aos de outras alturas; Qian et al. (2003), em menor escala, verificaram maior assentamento sobre biofilme da porção média da região entremarés em relação àqueles da porção superior ou inferior dessa zona.

No Brasil existem poucos estudos caracterizando costões rochosos e avaliando os fatores com influência na zonação (Ghilhardi et al. 2008), de forma que há padrões ainda não compreendidos. Em costões rochosos do sudeste, o mesolitoral superior usualmente é ocupado por cracas da espécie *Chthamalus bisinuatus* Pilsbry, 1916. enquanto ao mesolitoral médio pode ser dominado por ostras *Crassostrea brasiliana* Lamarck, 1819 e o mesolitoral inferior, ocupado por outra espécie de craca, *Tetraclita stalactifera* Lamarck, 1818 (Oliveira Filho & Mayal 1976). No entanto, em outros costões abrigados da mesma região, as faixas intermediária e inferior apresentam-se como faixas majoritariamente compostas por rocha nua, exceto pela presença de biofilme e de alguns indivíduos da craca *T. stalactifera* (Christofoletti et al. 2011), em um padrão de zonação aparentemente não observado em outras localidades. Desta forma, a mesma faixa de dominância poderia ser ocupada por biofilme (rocha nua) ou diferentes organismos macroscópicos, que limitam a distribuição inferior de *C. bisinuatus*. Assim, visamos avaliar a zonação do mesolitoral em diferentes escalas espaciais e testar dois possíveis fatores com influência nesse padrão de distribuição, o biofilme e a predação. Especificamente, os objetivos foram:

1) Analisar se as principais espécies (em termos de abundância e contribuição para a similaridade) dos costões rochosos estudados variam em abundância e em altura de ocorrência em três escalas espaciais (dentro de costão, entre costões e entre regiões),

2) Avaliar se ocorre recrutamento diferencial de cracas *C. bisinuatus* considerando a zona superior e a inferior do mesolitoral, e se a presença do biofilme dessas zonas influencia o recrutamento, revelando a influência de eventos pré-assentamento na zonação;
3) Avaliar se há mortalidade diferencial das cracas de acordo com a altura da zona de dominância no costão e se essa mortalidade é influenciada por predação, revelando a influência de eventos pós-assentamento na zonação.

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Capítulo 1

Spatial variation in midlittoral species distribution of subtropical sheltered rocky shores

Abstract

The study of spatial variation is important to theoretical and practical issues in community ecology, rising information on the local biodiversity, the patterns of species distribution and the processes that structure the assemblages. Rocky shores are an interesting model for these studies, as environmental gradients occur at small distances, making some patterns easy to visualize. In the present study we aimed to analyze the community variation in subtropical sheltered rocky shores (SE Brazil) at three spatial scales (within shores, between shores and between regions). To evaluate horizontal and vertical variation, we used a hierarchical sampling design with two regions and three shores in each region, which were sampled with vertical transects in two years. Community data were analyzed considering the upper, intermediate, and lower midlittoral zones with multivariate analyses, whereas the abundance of dominant species were analyzed with Mixed-Models Analysis of Variance. In community analyses, the main source of variation occurs between shores, but the abundance of dominant species varied mainly at smaller spatial scales, among transects within shores. The midlittoral presents zonation patterns, with differential dominance of species at different heights of the shore. Also, bare rock space was widespread, with high resource availability throughout the zones. We also found that processes that operate at small spatial scales strongly contribute to heterogeneity in these communities.

Keywords: Spatial variation, benthic community, midlittoral, subtropical sheltered rocky shores.

Introduction

Understanding the spatial and temporal components of species distribution is a fundamental matter in ecology, since this is correlated with species coexistence and diversity (Ricklefs 2004; Cottenie 2005). Thus, the study of variation in spatial or temporal scales for species or whole assemblages is an important tool to understand their structuring processes in diverse ecosystems (Brown et al. 1995; Beisel et al. 1998; Menge 1995).

Rocky shores are an interesting system for the study of scales of community variation, because environmental gradients occur even at small spatial scales (Underwood 2000). Variation in desiccation potential, which is modulated by tides and waves (Bustamante et al 1997), salinity (Mettam 1994; Westerbom et al 2002) and types of substrate (Hewitt et al 1998; Tanaka & Duarte 1998), combined with biotic interactions (Williams et al 2000; Hadfield & Paul 2001) and stochastic events (Pickett & White 1985; Berlow 1997; Harley & Paine 2009) constitute the main factors responsible for the patterns of species distribution in this environment. Therefore, the distribution, composition, and abundance of species in rocky shores might show spatial variation among patches (Menconi et al. 1999; Tanaka & Leite 2003), shores (Mc Quaid & Branch 1985; Bertness 2006) and regions (Bustamante & Banch 1996; Boaventura et al. 2002), and these patterns can also present vertical variation due to species zonation.

In rocky shores, zonation corresponds to a community vertical spatial variation. The rocky shore is usually divided in three zones, the supralittoral, the midlittoral and the infralittoral (see Sthephenson & Sthephenson (1949) and Lewis (1961, 1964) for more detailed classifications). Upper boundaries are mainly limited by physical factors such as desiccation potential, whereas biological factors, such as competition (Chapmam 1973; Branch 1984), predation (Menge 1976; Paine 1981), and facilitation (Bulleri 2009; Thomsen et al. 2010) have more variable influences and have more influence in the determination of the lower limits of species distribution (Connell 1961). Additionally, since many benthic organisms have indirect development and are subject to different mortality factors both in the larval phase and during settlement, recruitment rates also might influence the zonation patterns (Sutherland 1990; Pineda et al. 2002; Ank et al. 2009). All these factors influence vertical distribution, so that within each major zone other zonation patterns can be found (Connell 1972).

Sheltered rocky shores from southeastern Brazil show a midlittoral zonation pattern that apparently has not been observed yet for other places. In this shores, the midlittoral can be further subdivided in three zones: the upper midlittoral is usually dominated by barnacles *Chthamalus bisinuatus* Pilsbry, 1916, the intermediate midlittoral can be occupied by oysters *Crassostrea brasiliana* Lamarck, 1819, whereas the lower midlittoral is occupied by other barnacle species, *Tetraclita stalactifera* Lamarck, 1818 (Oliveira Filho & Mayal 1976). However, Christofoletti et al. (2011), working in Ubatuba (SE Brazil), found that the intermediate and low midlittoral zones can also be formed just by bare rock substrate, except for the presence of biofilm and scarce individuals of the barnacle *T. stalactifera*, although this pattern was found in only a few sites. Thus, we aimed to evaluate the vertical and horizontal spatial scales of variability in midlittoral communities of sheltered rocky shores at three spatial scales, regions, shores, and transects within shores. Specifically, we asked: 1) What are the dominant species in terms of abundance and contribution to similarity of the analyzed sites? 2) Are there differences at three spatial scales in the abundance of dominant species? 3) Do the main species occur at the same height of the midlittoral zone throughout the studied sites considering two years?

Material and Methods

Study sites

The study was carried out in two regions of the northern coast of São Paulo State, Ubatuba and São Sebastião, which spans about 100 km of shoreline in SE Brazil. The zonation pattern was first found in shores of Ubatuba and, as São Sebastião presents a similar shore line, it would be adequate to test the extension of the pattern. The climate is humid subtropical, without dry season and with hot summer (Cfa), according to Köeppen classification (Alvarez et al. 2014). Tidal amplitude is about 1.5 meters, with two high tides and low tides over a period of 24 hours.

São Sebastião Channel is formed between the city of São Sebastião, in the continent, and Ilhabela island, with widths varying between 2 and 7 km, and 22 km long. In this region we sampled the rocky shores in Barequeçaba (23º49’61.0”S 45º26’47.2”W), Julião (23º51’13.8”S 45º24’51.5”W) and Portinho (23º50’53.6”S 45º24’16.3”W). All these shores are protected from incoming waves by Ilhabela Island.

In Ubatuba we sampled rocky shores in Enseada (23º30’019”S 45º05’034”W), and Lamberto (23º27’10.1”S 45º02’76.9”W), which are sheltered from incoming waves by Ilha Anchieta, and Itaguá (23º30’0.0”S 45º05’02.0”), which is located in a closed bay also sheltered from wave action (Rocha et al 2010).

Sampling design

All shores were constituted by semi vertical (60-90°) granite rocky walls on which we deployed transects separated by approximately 3m. The number of replicate transects was 10 in 2013 and 6 in 2014, which were sampled in the autumn of both years. Along each transect, we photographed contiguous 10x10cm quadrats, using a digital camera. Photographed quadrats started in the upper end of the shore, usually marked by the presence of periwinkles, and continued down to the waterline.

From the photographs we identified the species to the lowest taxonomic level possible and measured the percentage cover of sessile organisms and bare rock using a grid (with 49 intersection point equally spaced) with Photoshop 7.0 software. We also counted the number of slow-moving animals, as limpets and predatory snails, directly from the photographs. Macroalgae were classified into functional groups following Steneck & Dethier (1994). Periwinkle abundance was determined directly in the field by counting number of individuals within 5x5cm plots placed within each sampling quadrat.

To calculate the center of each species distribution, we measured the height (in meters) of each quadrat along the transect with respect to the 0m tidal level. To evaluate the zonation within the midlittoral zone, defined as the zone between the upper distribution of chthamalid barnacles and the sublittoral fringe (which is dominate by macroalgae), we subdivided it in three equal zones (upper, intermediate, lower midlittoral zone), within each of which we deployed a similar number of quadrats. Thus, for each transect, we calculated mean cover or density of species within each of the three subzones.

Data analysis

To analyze the community variation at the studied scales, we compared the communities separately for each year. All data (percentage cover and density) were fourth-root transformed to enable comparisons (Underwood & Chapman 1998). Then, a similarity matrix based on Bray-Curtis similarity index was calculated, and data were ordinated with a Non-metric Multidimensional Scaling (NMDS) analysis. The effects of spatial variation (horizontal and vertical) on community composition were evaluated with a PERMANOVA model considering Regions and Levels (vertical zonation within the midlittoral) as fixed effects, and Shores within regions as a random effect, using PRIMER/PERMANOVA software (Anderson et al. 2008). Regions were considered a fixed effect because we wanted to compare another region with Ubatuba, where Christofoletti et al. (2011) found a different zonation pattern for the midlittoral at some sites. We used 999 permutations to obtain an α error rate = 0.05 (Manly 1997).

We analyzed the percentage of contribution of each species to similarity within sites and dissimilarity between sites, using Similarity Percentage Breakdown (SIMPER) analysis. To characterize the communities, we considered relevant variables with higher percentages of contribution in similarity and dissimilarity and with a cover higher than 10% across sites.

To evaluate patterns of spatial variation of the dominant species, we analyzed abundances with a Mixed-Model Analysis of Variance (ANOVA), with the same model described above to PERMANOVA. We transformed data to arcsine of square root (percentage cover) and natural logarithms (densities) to obtain homoscedasticity, following Underwood (1997). We evaluated the homoscedasticity of the samples with Cochran's test and, in some analysis, the variances were heterogeneous even with the data transformation. We proceeded with the analysis also in these cases since the ANOVA still robust with balanced designs and high number of replicates (Underwood 1997).

Additionally, we calculated the centroids of distribution of the dominant species. The centroids were defined as the mean height of occurrence of each species, considering the complete transect and not only the midlittoral zone. This was obtained by calculating weighted averages of the height sampled in the plots and the abundances of each taxon in percent cover or density. We analyzed taxa centroids with a Mixed-Model ANOVA, with Region and Year as fixed effects, and Shores within regions as a random effect. We expected a change in species' heights due to low precipitations and high temperatures in the summer between 2013 and 2014. The significance level of all analyses was $P < 0.05$.

Results

Zonation patterns in species composition

We found 24 taxa in the midlittoral of the studied sheltered rocky shores (Table I). According to the abundance measured and to SIMPER analysis, the main taxa characterizing the sampled shores were the barnacles *Chthamalus bisinuatus* and *Tetraclita stalactifera*, the mussels *Brachidontes* spp. (which included both *B. solisianus* and *B. darwinianus*), the oyster *Crassostrea* sp., the vermetid *Petaloconchus sp.*, articulated calcareous and crustose algae, and the herbivore gastropods *Nodilittorina lineolata* Orbigny, 1840 and limpets (included *Collisella subrugosa* and *Siphonaria hispida* ; since they were indistinguishable in the photos, they were grouped here) (TableI).

Table I. Biota and bare rock occurrence on the midlittoral of the rock shores sampled in 2013 and 2014. Data of frequency of occurrence in the transects per shore (in percentage). * High contribution in similarity within shores (≥10%) and dissimilarity between shores (≥5%) according to SIMPER analysis.

		São Sebastião Channel						Ubatuba					
Class	Taxon	Barequeçaba		Julião		Portinho		Enseada		Itaguá		Lamberto	
		2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
	Bare rock *	100	100	96.6	100	96.6	100	100	100	100	100	100	100
Cirripedia	*Chthamalus bisinuatus* *	63.3	66.6	70	61.1	73.3	61.1	56.6	61.1	46.6	38.8	46.6	38.8
	Tetraclita stalactifera *	63.3	33.3	60	44.4	46.6	38.8	66.6	44.4	73.3	61.1	50	61.1
Bivalvia	*Brachidontes* spp. *	46.6	55.5	3.3	22.2	16.3	11.1	0	0	10	11.1	6.6	0
	Crassostrea sp. *	63.3	50	0	5.5	6.6	5.5	0	5.5	10	0	6.6	5.5
Ascidiacea	*Didemnum perlucidum*	0	0	0	0	0	0	0	0	3.33	0	0	0
Demospongiae	*Hymeniacidon heliophila*	0	0	3.3	0	3.3	0	0	0	3.33	0	0	0
Sabellariidae	*Phragmatopoma* sp	0	0	0	0	0	0	0	0	0	0	3.33	0
Hydrozoa	*Sertularia marginata*	0	0	0	0	0	0	0	0	0	0	3.33	0
	Unidentified incrusting sp.	0	0	0	0	3.3	0	0	0	0	0	0	0
Macroalgae	Filamentous	3.33	0	0	5.5	3.3	0	6.6	5.5	6.6	11.1	6.6	5.5
	Foliose	0	0	0	5.5	3.3	0	0	0	10	11.1	3.33	0
	Corticated foliose	6.66	5.5	0	5.5	0	0	0	0	0	0	0	0
	Corticated macrophyte	3.33	0	0	0	3.3	5.5	0	0	6.6	0	6.6	0
	Leathery macrophyte	0	0	0	0	0	0	0	0	3.3	0	0	0
	Articulated calcareous *	16.6	5.5	26.6	16.6	20	16.6	10	0	33.3	33.3	6.66	5.5
	Crustose *	10	5.5	56.6	11.1	33.3	5.5	3.3	33.3	20	5.5	0	5.5
Gastropoda	*Petaloconchus* sp. *	0	11.1	0	16.6	13.3	22.2	20	5.5	20	11.1	20	22.2
	Nodilittorina lineolata *	23.3	50	26.6	50	36.6	33.3	33.3	38.8	36.6	44.4	26.6	0
	Littorina flava	0	0	0	0	10	0	3.3	0	6.66	5.5	10	38.8
	Stramonita haemastoma	0	0	3.3	0	3.3	0	0	5.5	3.33	0	0	0
	Morula sp	0	0	3.3	0	3.3	5.5	0	0	0	0	0	0
	Limpets *	60	22.2	0	0	16.6	11.1	6.6	0	33.3	16.6	0	0

There was a strong effect of vertical distribution on both years, with a significant interaction with shores in 2013, so that zonation patterns in community composition varied among shores within the same region in 2013, whereas in 2014 a more similar pattern was found within regions (Table II, Figure 1).

In 2013 the upper midlittoral was dominated by *C. bisinuatus* and *N. lineolata* in Ubatuba shores (with mean similarities within this zone in each shore varying between 77.7 and 91.1%, according to SIMPER analysis), but in São Sebastião Channel (SSC) only two of the shores presented this dominance; in Barequeçaba, *N. lineolata* densities were too small to contribute to mean similarity among samples of this zone. Mean similarities within this zone in SSC shores varied between 83.1 and 88.7%.

The intermediate midlittoral was dominated by *T. stalactifera* in most shores. In Ubatuba, two species contributed to most similarity in Enseada (*T. stalactifera* and *C. bisinuatus*; similarity = 79.0%) and Itaguá (*T. stalactifera* and limpets; similarity = 66.7%), whereas in Lamberto only *T. stalactifera* dominated this zone (similarity = 70.4%). In SSC, a distinct pattern was found for each shore: in Portinho, only *C. bisinuatus* dominated (similarity = 66.0%), in Julião both *T. stalactifera* and *C. bisinuatus* dominated (similarity = 77.0%), whereas in Barequeçaba 5 taxa co-dominated this zone: *T. stalactifera*, *C. bisinuatus*, *Brachidontes* spp., *Crassostrea* sp., and limpets (similarity = 75.2%).

The lower midlittoral was dominated by *T. stalactifera* in all shores sampled, but other taxa also contributed strongly to similarity patterns within each shore. In Ubatuba, *Petaloconchus* sp. contributed in Enseada (similarity = 70.2%) and Lamberto (similarity = 57.1%), whereas articulated calcareous algae contributed in Itaguá (similarity = 63.9%), whereas in SSC articulated calcareous and incrusting algae contributed to dominance in Julião (similarity = 81.6%) and Portinho (similarity = 57.1%), whereas in Barequeçaba *Brachidontes* spp., *Crassostrea* sp., and limpets contributed to similarity patterns within this zone (similarity = 71.7%). Cover of bare rock was higher in the upper and intermediate midlittoral, with values above 37.8%.

Table II. Results of Mixed-Effects PERMANOVA comparing composition of assemblages in the lower, intermediate, and upper midlittoral of shores within São Sebastião Channel and Ubatuba, in 2013 and 2014. ** $P < 0.01$.

Source of variation	df	MS	*F*
2013			
Region: *R*	1	11396	2.2
Level: *L*	2	48792	33.6**
$R \times L$	4	5057.4	11.7**
Shore(Region): *S(R)*	2	4162.9	2.87**
$L \times S(R)$	8	1450.2	3.3**
Error	162	430.72	
2014			
Region: *R*	1	6784.9	3.2
Level: *L*	2	21569	27.2**
$R \times L$	4	2103.1	3.8**
Shore(Region): *S(R)*	2	1309.7	1.6
$L \times S(R)$	8	793.92	1.4
Error	90	547.8	

In 2014, zonation patterns were more similar within regions, and only a significant Region x Level interaction was recorded (Table II). SIMPER analysis indicated that mean similarity within each level was lower than recorded in 2013. In Ubatuba, only *C. bisinuatus* contributed to the upper midlittoral (similarity = 84.3%), both *T. stalactifera* and *N. lineolata* contributed to the intermediate zone (similarity = 65.5%), and both *T. stalactifera* and incrusting algae contributed to the lower midlittoral (similarity = 70.7%). In SSC, both *C. bisinuatus* and *N. lineolata* contributed with the upper (similarity = 82.8%) and intermediate zones (similarity = 56.3%), whereas *T. stalactifera* and *Petaloconchus* sp. contributed with the lower midlittoral (similarity = 69.8%)

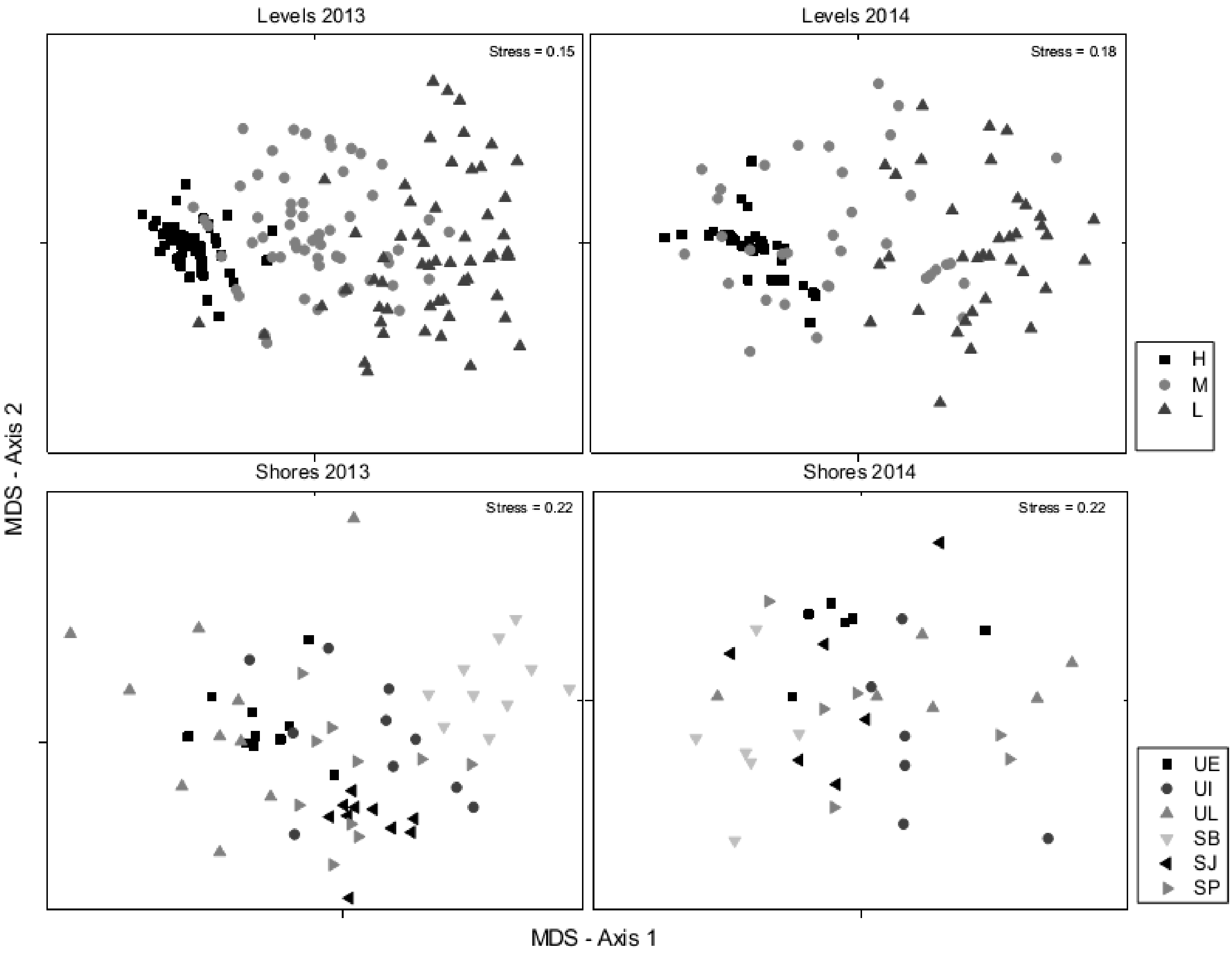

Figure 1. Multidimensional Scaling (MDS) ordination considering the three midillitoral zones and considering the average species abundance per shore H = high, M = middle, L = low shore; U = Ubatuba; S = São Sebastião regions; E = Enseada, I = Itaguá, L = Lamberto, B = Barequeçaba, J = Julião, P = Portinho shores.

Spatial and temporal variation in abundances of main taxa

Chthamalus bisinuatus dominated the upper midlittoral in both regions, although with higher values in SSC, resulting in a significant interaction between Level and Region in 2013 and a trend in 2014 ($P = 0.053$; Table III). In the lower midlittoral, cover was very low and similar for both regions, whereas a different pattern was found between years in the upper midlittoral (Figure 2). In 2013, a significant higher cover was found in the high midlittoral when compared with the intermediate zone (Tukey´s HSD test, $P < 0.05$), but in 2014 no significant differences were found. Patterns were consistent within regions, with no significant differences among shores (Table III)

Table III. Results of Mixed-Effects ANOVA comparing taxa abundances in the midlittoral of shores within São Sebastião Channel and Ubatuba, in 2013 and 2014. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

		Chthamalus		*Tetraclita*		*Brachidontes*		*Crassostrea*		*Petaloconchus*	
Source of variation	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
2013											
Region: R	1	1.052	28.8**	0.248	4.4	0.318	1.1	0.320	0.9	0.092	3.3
Level: L	2	4.075	137.8***	2.701	40.6***	0.123	15.	0.235	1.5	0.265	7.2**
$R \times L$	2	0.372	12.6**	0.068	1.0	0.091	1.1	0.130	0.8	0.072	1.9
Shore(Region): $S(R)$	4	0.037	1.24	0.056	0.8	0.285	3.5	0.154	2.3	0.027	0.7
$L \times S(R)$	8	0.030	0.97	0.066	2.2**	0.080	6.4***	0.013	11.6***	0.037	3.7**
Error	162	0.030		0.030		0.012				0.010	
2014											
Region: R	1	1.476	13.2*	0.213	3.5	0.341	3.1	0.061	0.6	0.005	0.2
Level: L	2	2.033	42.1***	1.037	34.6***	0.103	3.2	0.43	1.9	0.242	11.1**
$R \times L$	2	0.210	4.35	0.058	1.9	0.066	2.0	0.011	0.5	0.001	0.06
Shore(Region): $S(R)$	4	0.112	2.31	0.061	2.0	0.110	3.4	0.095	4.3**	0.022	1.0
$L \times S(R)$	8	0.048	1.31	0.030	1.8	0.032	1.7	0.022	2.1**	0.022	1.5
Error	90	0.037		0.017		0.018		0.010		0.15	

[a] $P = 0.05$, * $P < 0.05$, ** $P < 0.01$

		Articulated algae		Crustose algae		Bare rock		*Nodilittorina*		Limpets	
Source of variation	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
2013											
Region: R	1	0.020	0.2	0.782	2.1	2.677	13.6**	0.505	0.6	2.639	0.4
Level: L	2	0.731	12.2**	0.758	5.7**	1,323	12.2**	49.590	40.4***	3.163	2.4
$R \times L$	2	0.019	0.3	0.420	3.2	0.097	0,90	1.989	1.6	0.913	0.7
Shore(Region): $S(R)$	4	0.086	1.4	0.361	2.7	0.196	1.8	0.818	0.6	5.465	4.2**
$L \times S(R)$	8	0.060	2.8**	0.132	9.6***	0.109	1.9	1.225	1.8	1.300	6.8***
Error	162	0.021		0.014		0.056		0.677		0.190	
2014											
Region: R	1	0.004	0.0	0.091	1,0	1.597	19.6	2.436	1.6	0.004	0,0
Level: L	2	0.188	2.2	0.153	4.3	0.015	0.1	27.826	30.8***	0.172	1.3
$R \times L$	2	0.006	0.0	0.075	2.1	0.263	1.8	3.205	3.5	0.022	0.1
Shore(Region): $S(R)$	4	0.022	1.0	0.079	0.9	0.090	2.5	1.487	1.6	0.148	1.1
$L \times S(R)$	8	0.022	1.5	0.082	3.1**	0,035	1.5	0.903	0.6	0.132	1.2
Error	90	0.15		0.026		0,023		1.328		0.106	

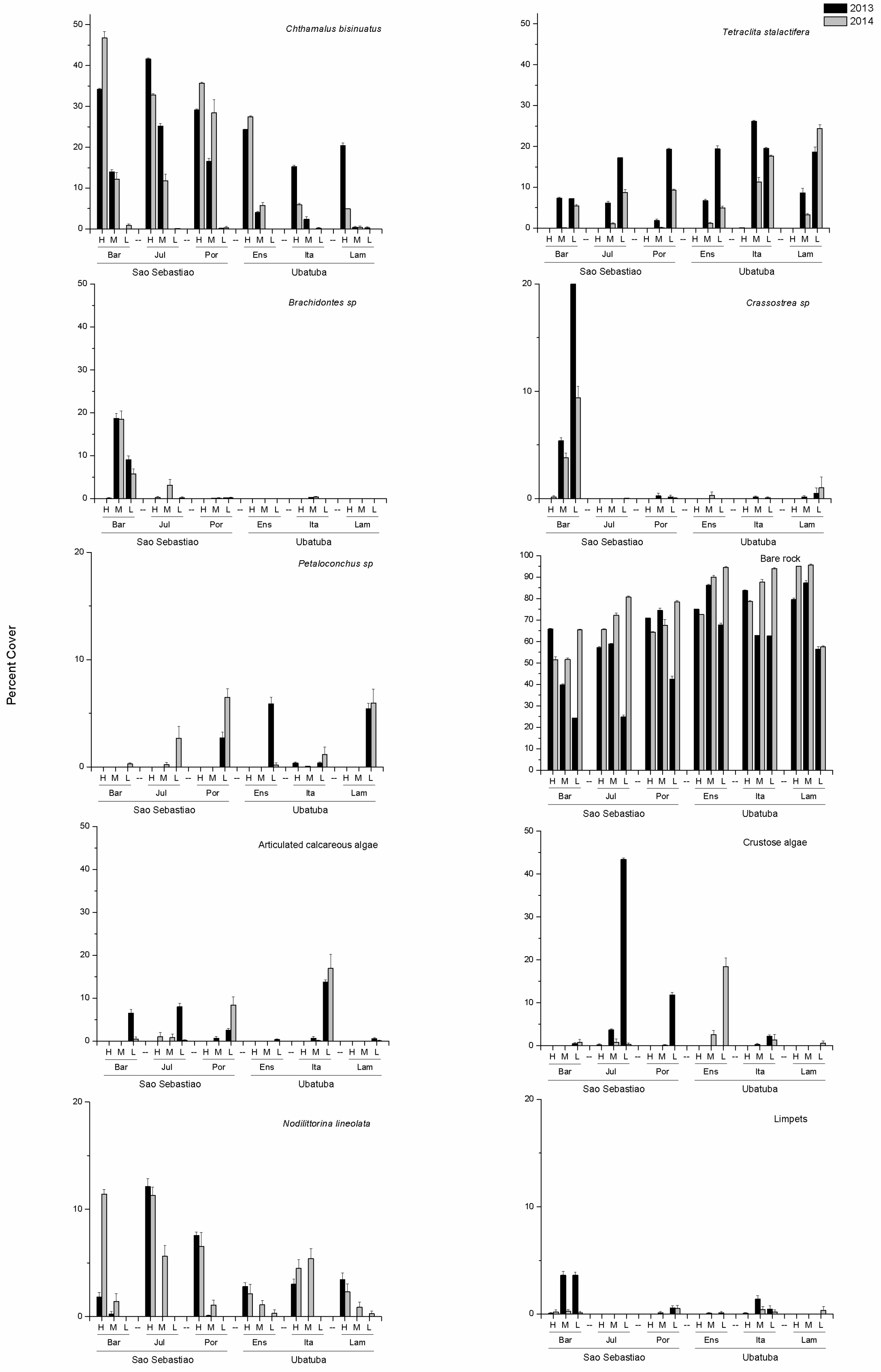

Figure 2. Species zonation across the sheltered rocky shores in both sampled years. Bar = Barequeçaba, Jul = Julião, Por = Portinho (São Sebastião shores); Ens = Enseada, Ita = Itaguá, Lam = Lamberto (Ubatuba shores).

The other barnacle species recorded, *T. stalactifera*, occurred mainly in the low midlittoral, with some occurrence in the intermediate zone. Patterns differed among shores within regions (Table III, Figure 2) in 2013, but in 2014 only the level effect was significant, with no other spatial effects (Table III). Cover was significantly higher in the lower midlittoral when compared with the intermediate zone, which was significantly higher than in the upper zone, where scant individuals were found (Tukey´s HSD test, $P < 0.05$). Bivalves presented a more patchy distribution, with significant interactions between level and shores within regions, except for *Brachidontes* in 2013 (Table III). *Brachidontes* spp. had higher cover in the intermediate midlittoral, mainly in Barequeçaba (SSC), whereas in Ubatuba, there was some cover only in Itaguá (Figure 2). Oysters (*Crassostrea* sp.) also presented higher cover in Barequeçaba, but in the lower midlittoral, with scattered distributions in the other shores in both regions (Figure 2).

Petaloconchus sp. occurred mainly in the lower midlittoral, although a widespread distribution of this taxon was found in Itaguá (Figure 2). In 2013 there was large variation among shores within regions (Table III), and only in Julião (SSC) this taxon was not found (Figure 2). On the other hand, in 2014 it occurred in all sampled shores, with only a significant level effect (Table III). Cover was significantly higher in the lower midlittoral (Tukey´s HSD test, $P < 0.05$), and very low cover was found in the intermediate and upper zones (Figure 2).

Macroalgae also occurred mainly in the lower midlittoral, but with different patterns among shores within regions (Table III). Articulated calcareous algae were present mainly in the lower midlittoral, whereas crustose algae also occurred mainly in the lower zone, with some cover in the intermediate midlittoral (Figure 2).

Limpets and periwinkles presented distinct vertical distributions. *N. lineolata* was more concentrated in the high and intermediate midilittoral (Table III), with no effects of shores and regions (Figure 2). Limpets were found in higher abundance in the intermediate and lower midlittoral. They were absent in Julião (SSC), resulting in significant variation among shores within regions in 2013 (Table III). In 2014, limpet abundances were lower, with no records in Julião and Enseada (Ubatuba), and no significant effects on abundances were recorded (Table III, Figure 2).

The amount of available bare rock was high along the shores studied. In 2013, only the main effects of Level and Region were significant (Table III), with lower availability in the lower midlittoral, and in SSC shores when compared to Ubatuba

(Figure 2). In 2014, no significant effects were found, with availability of bare rock among shores and levels, in both regions studied (Table III, Figure 2).

The variance components analysis indicated that there was a large variation among transects within shores for all variables evaluated, and this factor accounted for 38.2 – 100.0% of the variation in 2013, and 67.7 – 79.6 in 2014 (Table IV). Shore(Region) and the interaction Level x Shore(Region) also contributed to variation in *Brachidontes* (26.9 and 26.9%, respectively), *Crassostrea* (206 and 41.2%), crustose algae (23.5 and 35.3%), and limpets (31.7 and 25.3%) abundances in 2013, but in 2014 these factors presented much lower contributions to variance components (Table IV).

Table IV. Variance components for random effects for each year analyzed (values in percentage).

Variable	Shore(Region)		Level*Shore(Region)		Within shore	
	2013	2014	2013	2014	2013	2014
Bare rock	4.7	4.8	7.8	27.4	87.5	67.7
Chthamalus bisinuatus	0	9.3	0	4.7	100	86.0
Tetraclia stalactifera	0	9.53	11.8	9.5	88.2	81.0
Brachidontes sp	26.9	16.7	26.9	8.3	46.2	75.0
Crassostrea sp	20.6	9.5	41.2	9.5	38.2	81.0
Petaloconchus sp	0	0	23.1	6.3	76.9	93.8
Articulated calcareous algae	3.8	0	15.4	25.7	80.8	74.3
Crustose algae	23.5	10.7	35.3	7.1	41.2	82.1
Nodilittorina lineolata	0	2.4	7.5	0	92.5	97.6
Limpets	31.7	0.9	25.3	3.6	43.0	95.5

Centroids: patterns of spatial and temporal variation of species on shore heights

When using the centroid method, we could determine the center of the distribution of each taxon evaluated, by weighting the height of occurrence by the percent cover of each taxon. Thus, we could evaluate patterns of vertical zonation of each taxon directly on each shore (Figure 3). For example, both barnacle species occur in very different vertical heights: *C. bisinuatus* did not present differences in mean heights among the studied shores in both years (Table V), occurring at about 0.96m from the 0.0m level. On the other hand, mean height of *T. stalactifera* decreased from 2013 to 2014 (Table IV), from about 0.6 to 0.5m, and was lower in SSC when compared to Ubatuba (Table IV). Mussels and oysters presented distinct vertical distributions according to the shore studied, occupying higher portions in Barequeçaba (Table IV, Figure 3). The vermetid *Petaloconchus* sp. did not present variation in vertical height among sites and years (Table V), occurring about 0.16m above the 0.0m level (Figure 3). Macroalgae presented different results: articulated calcareous algae did not present variation in vertical height among sites and years (Table V), with the center of its distribution at about 0.25m, whereas crustose algae presented an interaction between Year and Shores nested in Regions (Table V), with different patterns of variation between years for each shore studied (Figure 3). Periwinkles (*N. lineolata*) preferentially occurred in the upper midlittoral in all shores and years (Table IV), at a mean height of about 1.0m (Figure 3). Finally, in the shore where more limpets were recorded, they presented a wider distribution (Barequeçaba in SSC, Itaguá in Ubatuba), resulting in differences in the average height of occurrence between shores (Table V, Figure 3).

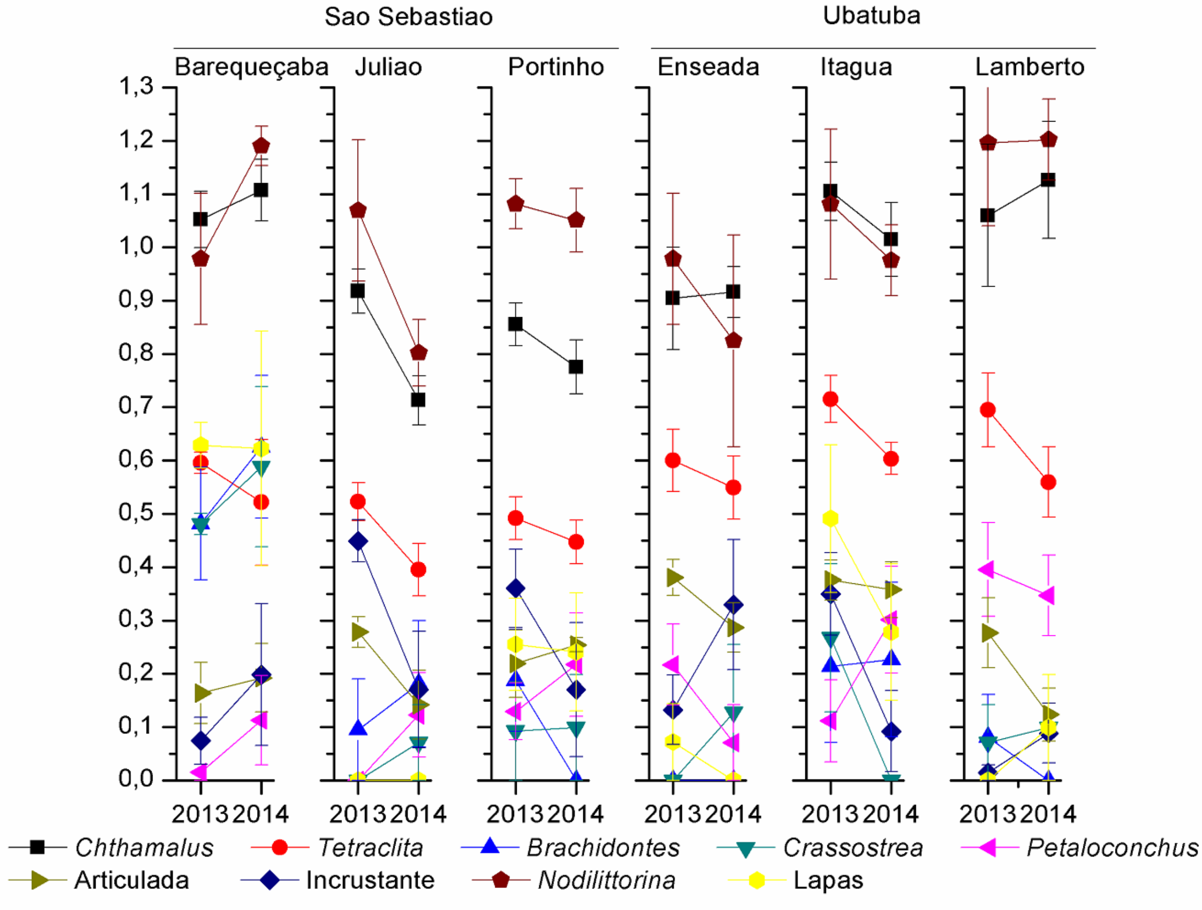

Figure 3. Centroids or weighted average of occurrence to the main benthic species found in each shore and year.

Table V. Results of Mixed-Effects ANOVA comparing taxa height (centroids) in the midlittoral of shores within São Sebastião Channel and Ubatuba, in 2013 and 2014. ** *P* < 0.01.

		Chthamalus		*Tetraclita*		*Brachidontes*		*Crassostrea*		*Petaloconchus*	
Source of variation	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Region: *R*	4	0.311	1.2	0.351	9.2**	0.688	1.1	0.368	0.6	0.446	3.3
Year: *Y*	4	0.037	0.8	0.185	26.8**	0.000	0.0	0.003	0.0	0.058	1.0
R × *Y*	4	0.030	0.6	0.002	0.2	0.008	0.1	0.055	0.6	0.061	1.0
Shore(Region): *S(R)*	4	0.246	5.6	0.036	5.2	0.586	9.2**	0.567	6.7**	0.134	2.3
Y × *S(R)*	84	0.044	0.8	0.007	0.2	0.063	0.8	0.84	1.4	0.056	0.2
Error	84	0.048		0.024		0.079		0.58		0.040	

		Articulated algae		Crustose algae		*Nodilittorina*		Limpets	
Source of variation	df	MS	*F*	MS	*F*	MS	*F*	MS	*F*
Region: *R*	4	0.183	3.8	0.155	0.3	0.071	0.0	0.407	0.4
Year: *Y*	4	0.074	2.2	0.062	1.3	0.006	0.5	0.026	0.6
R × *Y*	4	0.021	0.6	0.122	0.7	0.017	0.1	0.017	0.4
Shore(Region): *S(R)*	4	0.048	1.4	0.119	0.7	0.214	1.7	1.037	22.6**
Y × *S(R)*	84	0.033	1.3	0.166	3.4**	0.119	0.8	0.046	0.7
Error	84	0.025		0.048		0.138		0.067	

Discussion

In a general way, we found that the variability in the communities of the subtropical sheltered rocky shores sampled was more related with the abundance than with the composition of species horizontally or across shores, while the patterns of presence and absence of species was more remarkable vertically, or across zones. Thus, the nature and the magnitude of community variation might change according to the source of variation, being necessary to consider both the vertical and horizontal spatial sources of variation to study spatial ecology on rocky shores. Other important factor to be considered in these communities is the scale of analysis, since the patterns of species distribution are not scale independent (Menge & Oslo 1990; Underwood & Chapmam 1996). An example occurs in a study in Mediterranean rocky shores, which showed more vertical than along-shore variation at small (until hundreds of centimeters) but not at larger spatial scales (Benedetti-Cecchi 2001). As exposed in Valdivia et al. (2014), in the present study the relation between horizontal and vertical spatial scales in community variability is demonstrated through the significant interactions between level and the smaller spatial scale of the hierarchical design.

The assemblages of the sheltered rocky shores sampled present vertical spatial variation, since we found significant results in variability between levels and/ or in their interactions with the horizontal scale of variation, showing general patterns of zonation within the midlittoral zone. The occurrence of subzones is widely related to rocky shores across different localities, in temperate (Boaventura 2002) or tropical (Lubchenco et al. 1994) environments and in sheltered or exposed regimens (Bertness et al 2006), due to species tolerance and interactions in a fine scale of environmental conditions. Thus, the present work supports the occurrence of subzones in Brazilian southeastern sheltered shores.

The significant scale of horizontal variability to communities, in general, and to the most part of the taxa, individually, occurs between shores. Besides that, a high part of the variation is represented within shores. Similar results were found in other studies with rocky substrate, where the higher variation occurs at the scales of centimeters to meters (Underwood & Chapaman 1998, Beneditti-Cecchi 2001, Olabarria and Champman 2001). Fraschetti et al. (2005) suggest that the small scale of variation is a general property of marine coastal assemblages, as well as in other environments with complex interactions between physical and biological processes.

A larger scale of spatial variability was found to the barnacle *Chthamalus bisinuatus*, which showed higher cover in SSC shore than in Ubatuba. A peculiar result was found to the barnacle *Tetraclita stalactifera* as well, since it presented a higher height of occurrence in Ubatuba than in São Sebastião, decreasing from 2013 to 2014. According to a survey on native and introduced barnacles in Brazil, *C. bisinuatus* is the most widespread and abundant barnacle in the upper and intermediate intertidal zones of 26 shores sampled across the country's coast (Klôh et al. 2013). This same study shows that *T. stalactifera* is found mostly in the medium and low intertidal zones and are absent only in the extremes latitudes sampled. Our results corroborate this pattern, since the barnacles are present in mentioned zones of all sampled sites. Notwithstanding, in spite of the broader distribution, barnacle populations can be influenced by some factor that decreases the abundance of *C. bisinuatus* and restricts the spatial distribution of *T. stalactifera* regionally.

Considering that *C. bisinuatus* is more abundant in the region where *T. stalactifera* has a narrower distribution, our first hypothesis to this pattern is the existence of competition between these species. This interaction has been considered a major negative influence on barnacle colonization (see review in Underwood 2000) and, therefore, might determine the vertical limits between the species (Connel 1961). However, bare rock is a major component of the shores sampled, and empty spaces are available even in the intermediate midlittoral, the region of co-occurrence of these barnacles, making this hypothesis unlikely. Actually, the lower abundance of *C. bisinuatus* or the lower occurrence of *T. stalactifera* in Ubatuba was not accompanied by a higher abundance or distribution of any competitor, and bare rock was the only variable with changes at the same spatial scale.

Predation is another biological factor that might influence the community structure in a general way, including barnacle colonization (Paine 1981; Navarrete & Castilha 2003), and this could be consistent with the amount of bare rock found. In our study, the predators found were the gastropods *Stramonita haemastoma* and *Morula sp.*, but in insufficient abundance to proceed with analyses of spatial variability, although correlation with some of the barnacle results could explain the patterns. Barnacle mortality by biological factors might also be caused by conspecific predation (Navarrete & Wieters 2000) or by the indirect effect in removing barnacle recruits, known as a bulldozing effect, as caused by limpets (Gateno et al. 1996; Menge et al. 2010). Our methods allow making inferences on the second hypothesis; however, again, there is no variation in limpet occurrence to support this hypothesis.

Changes in barnacle abundance on regional scales might also be related with abiotic, environmental factors. Temperature, for example, could influence barnacle distribution, since they usually live close to lethal environmental temperatures within its geographical distribution (Foster 1969). A study on temperate rocky shores showed that colonization of *Semibalanus balanoides* changes at a regional scale due to thermal stress (Leonard 2000). This may not be the case here, considering the same climate pattern in both regions, but temperature could influence temporal changes in abundances. Chemical factors may also determine the distribution of rocky shore populations, and consequently the community composition. A study carried out in Brazil found that *C. bisinuatus* and *T. stalactifera* are absent from areas with low salinity and high sewage pollution, and also that these polluted areas have more unstable communities (Junqueira et al. 2000).

The main scale of spatial variation, which occurs within and between shores, might be influenced mostly by the factors listed above. Furthermore, wave action intensity can influence the communities at a range of spatial scales, even considering close areas (Ballantine 1961; Bustamante & Branch 1996). Accordingly, the differential wave action can influence the observed patterns. Although all sampled shores are sheltered by wave action, some exposure variation might occur across shores and even at smaller distances, resulting in lower abundances of filter-feeding animals in the areas with lower hydrodynamics (Underwood 1981; Menge & Farrel 1989).

In the subtropical sheltered shores studied there is widespread dominance of specific taxa in the zones within the midlittoral, as discussed above, and also low abundance of sessile organisms throughout the midlittoral zones, with high availability of bare rock for colonization. Our data supports the observation that cover of bare rock is high in these shores, although not only in a determined zone, as also found by Christofoletti et al. (2011). However, is important to mention that, in the present study, this bare zone could be slightly diluted because of the sampling method. Sampling in interrupted vertical transects (using juxtaposed plots) is not so efficient to emphasize clear limits between the subzones. On the other hand, this is the best option to sample all the rock vertical variability avoiding skewness.

Studying spatial patterns has been a major topic in ecology, and the development of these studies includes challenges in achieving adequate methods to sample and analyze data considering the particularities of each environment (McIntire & Fajardo 2009). The present study contributes with a new approach to describing species vertical heights in intertidal zonation patterns, and also describes patterns of species abundance along subtropical sheltered rocky shores, identifying the main source of spatial variability considering the three spatial scale analyzed, both horizontal and vertically. In this way, the structuring processes in these benthic communities also act at these (small) spatial scales, and interactions among processes can contribute to this variation. This information is important to evaluate the status of biodiversity in the sampled shores, generating a reference framework about the studied system, subsiding studies on environmental impacts.

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Capítulo 2

Recruitment and differential post-settlement mortality in barnacle population of subtropical sheltered rocky shores

Abstract

The structure of biological communities is determined by population recruitment and mortality rates. Rocky shore species generally have a settlement phase, leaving the water column to occupy a benthic position in the rock. Beyond this, species are subject to a heterogeneous environment, including stressful conditions and several biological interactions, which may cause the mortality of a large number of individuals, influencing recruitment rates. In thisstudy, we evaluated experimentally the roles of differential barnacle recruitment and of differential mortality in influencing a zonation pattern in subtropical sheltered rocky shores of southeastern Brazil, asking why there is almost no barnacles in the lower midlittoral zone. In these shores, the upper midlittoral is dominated by the barnacle *Chthamalus bisinuatus*, whereas the intermediate and lower midlittoral is formed basically by bare rock, with no sessile dominant species. In order to evaluate the differential recruitment, which could be influenced by the biofilm, we collected data using a digital camera modified to take photos in infrared wave-lengths, through which we could visualize possible barnacle recruits and also calculated a Normalized Difference Vegetation Index (NDVI) to measure the density of photosynthetic microorganisms. To evaluate the differential barnacle mortality, which could be influenced by predation, we colonize plots with *C. bisinuatus* and then transplant the half of the plots to the lower midlittoral, following the survival of each barnacle through photos. We also sampled the abundance of predators considering both zones in these shores. The results showed that the barnacle recruitment was low even in the upper as in the lower midlittoral during all the exposure time. The biofilm was more abundant in the lower than in the upper midlittoral and the barnacle mortality rate is also higher in the lower than in upper shore, probably due high predation. Thus, the zonation pattern studied should be a result of combined structuring factors, with significant participation of the barnacle differential mortality in the low midlittoral zone.

Keywords: Barnacle, recruitment, biofilm, differential mortality, zonation, sheltered rocky shores.

Introduction

Patterns of species distribution in open assemblages depend ultimately on recruitment, migration and mortality rates of populations component. Recruitment and mortality may be directly correlated, for example when a higher population size leads to greater competition, predation or parasitism, i.e. density-dependent process (see Hassel 1986 for a review). Notwithstanding, mortality events may also be independent of population number or recruitment rates, as occurs in the relation between prey recruitment and predation by broadcasting predators (Wieters et al. 2008) or in the mortality by the action of stressful environmental conditions (Chan et al. 2006). Thus, patterns in communities depend on location-specific factors, life history traits and even on the scale analyzed (Methatta & Petraits 2008).

As many of benthic species on rocky shores have indirect development, including a pelagic phase before the benthic stage, the settlement process is an important phase in recruitment (Jenkins et al 2000). Therefore, recruitment variability is related to larval pool sizes (Sutherland 1990), to transport processes (Gaines & Bertness 1992) and to characteristics of the substrate, including its type, texture and heterogeneity, as these factors may influence the larval choice (Chabot & Bourget 1988, Hills & Thomason 1996, Tanaka & Duarte 1998). The presence of some organisms may also determine settling decisions because benthic species may avoid direct interference, as in preemption competition (Dayton 1971), or avoid predation by means of chemical cues signaling (Johson & Strathman 1989). Thus, differential species recruitment may be responsible for generating zonation patterns in rocky shore communities (Grosberg 1982, Pineda et al. 2002).

Biofilms are considered pioneering organisms in the occupation of bare substrates in rocky shores (Whal 1989). Consequently, the settlement of macro organisms occurs on this complex of microorganisms and may be influenced by it (Lau et al 2005). Although some studies found that biofilms have positive influences in the recruitment of sessile invertebrates (Hadfield & Paul 2001, Clare 1996), others found that biofilms may inhibit fouling by macroorganisms (Wieczorek & Todd 1997, Oliver et al. 2000). These different responses may occur because biofilms can vary spatially and temporally, with varying densities and compositions (Thompson et al. 2005, Thiyagarajan 2010). As this variation may occur at small spatial scales, such as at different heights on the shore (Thompson et al. 1998), biofilms can also influence zonation patterns. For example, the barnacle *Balanus amphitrite* has high settlement rates on biofilms in the mid-intertidal compared to biofilms present in high or low intertidal zones (Qian 2003).

Patterns in the distribution of rocky shore communities are also influenced by differential mortality of the belt-forming organisms. The several limiting factors in the environment, such as desiccation stress caused by the action of tides, may increase mortality rates of some species according to the height on the shore, even in the adult phase (Delany et al 2003). Negative biological interactions, including smothering by competing species and high predation rates in different areas, may also lead to vertical patterns of species distribution (Connell 1961). A classical study in the intertidal zone illustrated the importance of mortality in determining zonation pattern, showing that the restricted occurrence of the barnacle *Balanus glandula* in the upper zone was not dependent on recruitment, as this species settles in all zones, but by predation, which eliminates the barnacle from the intermediate and lower zones (Connell 1970). The same pattern was found for the barnacle *Chthamalus stellatus,* whose distribution is limited in the lower shore by higher competition and predation, whereas differential recruitment was not observed (Benedetti-Cecchi et al. 2000).

In this study we evaluated the roles of recruitment and post-settlement mortality in chthamalid barnacle distribution, process that may influence zonation patterns in subtropical sheltered rocky shores in southeastern Brazil. In some shores of this region, the upper midlittoral is dominated by barnacles *Chthamalus bisinuatus* Pilsbry, 1916, whereas the intermediate and lower midlittoral zones may be composed solely by bare rock substrate, except for the presence of biofilm and scarce individuals of the barnacle *Tetraclita stalactifera* Lamarck, 1818 (Christofoletti et al. 2011). As bare space is the only conspicuous element in the intermediate and lower midlittoral, we tested whether there is differential barnacle recruitment and differential barnacle mortality in these zones, assessing which processes are structuring these populations and consequently, the rocky shore communities. Specifically, we aimed to answer the following questions: 1) Is the recruitment of *C. bisinuatus* lower in the lower midlittoral than in the upper midlittoral? 2) Does biofilm influences the recruitment of *C. bisinuatus*? 3) Do biofilm abundances vary with height on the midlittoral zone? 4) Is the mortality rate of *C. bisinuatus* higher in the lower midlittoral than in upper midlittoral? 5) Are barnacle predators more abundant in the lower than in upper midlittoral?

Material and Methods

Study site

The study was carried out in Ubatuba, city of the SE Brazil. The climate is humid subtropical, without dry season and with hot summer (Cfa), according to Köeppen classification (Alvarez et al. 2014).

The experiments were carried out in the shores of Lamberto (23º30’0.0”S 45º05’02.0”) and Enseada (23º30’019”S 45º05’034”W). Both rocky shores are formed by granite boulders and are sheltered of wave action.

Experimental design

Effects of biofilms on barnacle recruitment

To evaluate the influence of biofilms on barnacle recruitment, we carried out a factorial experiment (2 x 3) in randomized blocks, with the factors Height (upper and lower midlittoral) and Biofilm (absent, biofilm from the upper midllitoral, biofilm from the lower midlittoral) in Lamberto shore. We used five experimental blocks; each block was formed by six granite plates (10x10 cm) fixed on the rocky shore, three located in the upper midlittoral (or zone of the barnacle *Chthamalus bisinuatus*) and three in the lower midlittoral (or zone of bare rock), totalizing 30 plots. The experiment was carried out in mid-2014.

After a period of 60 days, we would evaluate the effects of biofilms on barnacle recruitment by manipulating the granite plates: 1) exchange one plate from the lower midlittoral to the upper midlittoral and vice-versa; 2) scrape and burn one plot in each zone; 3) leave an un manipulated plot in each zone (control). Thus, in each zone we would have one plot with biofilm from the same zone, one plot with biofilm from the other zone being tested, and one plot without biofilm (Figure 4). This would enable to test directly the influence of biofilm from different heights on barnacle recruitment, since different characteristics of biofilm should be related with the heights in which it was developed. Unfortunately, we could not conclude the experiment, because of low barnacle recruitment during all experimental duration (see Results).

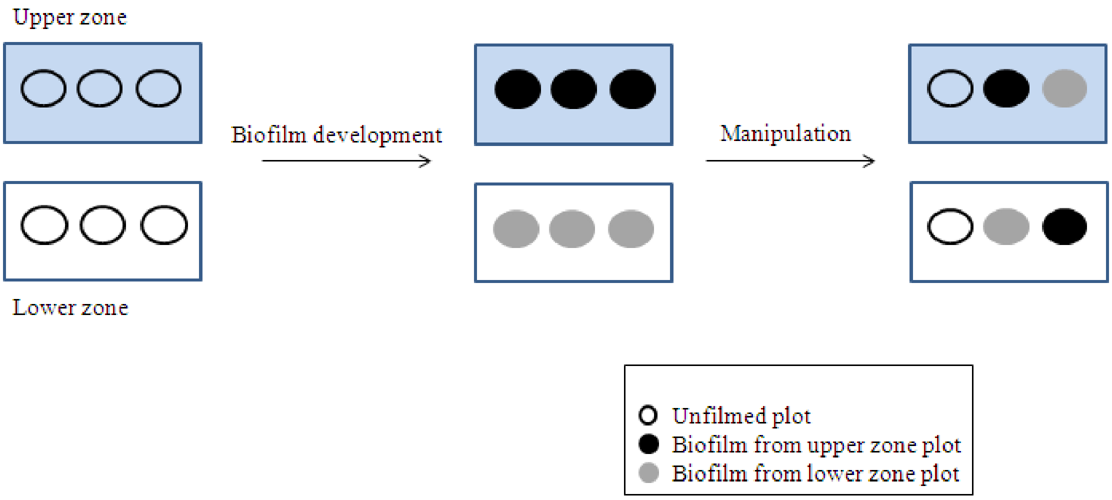

Figure 4. Experimental design to test the biofilm influence on recruitment of the barnacle *Chthamalus bisinuatus*.

To evaluate the development of biofilm on different heights, we photographed the experimental plots after 5, 9, 15, 40, 60, 200 days, with a Canon ELPH 110 HS 16.1 digital camera, modified to take images in infrared. Thus, we could analyze the development of microphytobenthic biofilm by calculating the Normalized Difference Vegetation Index (NDVI; Rouse et al. 1973), based on pixel values in the infrared and red channels. This index considers the ratio between the near infrared wave-length (NIR – scattered by the photosynthetic cells) and the red wave-length (absorbed by photosynthetic cells; see equation 1) and is directly proportional to the concentration of chlorophyll on rock surfaces (Murphy et al. 2005, 2006, 2009). The light condition was standardized using a photo diffuser and a pattern of reflectance when the photos were taken. The photos were processed using a script in the software Matlab, considering the following equation:

$$\text{NDVI} = (\text{NIR} – \text{red})/ (\text{NIR} + \text{red})$$

In which *NIR* is the reflectance of image captured in the near infrared bands and *red*, is the reflectance of image captured in the red band. *NDVI* values range between -1 to 1; zero values indicating no photosynthetic cover, positive values corresponds to presence of photosynthetic cover and negative values responds to high reflectance, such as caused by water.

Differential barnacle mortality

We evaluated barnacle mortality at different heights on the shore with a randomized blocks experiment with one factor, Height (upper and lower midlittoral). We used seven experimental blocks in each of two shores (Enseada and Lamberto), where each block contained a pair of plots, one located in the upper midlittoral (or zone of the barnacle *Chthamalus bisinuatus*) and one in the low midlittoral (or zone of bare rock), totalizing 28 plots. The experiment was carried out in early 2014.

We fixed all PVC plates (10x10cm) in the upper midlittoral of an exposed rocky shore in São Sebastião, where *Chthamalus bisinuatus* dominates, to allow colonization and, after 60 days, we transferred the plates to the experimental shores in Ubatuba. The plates presented a variable number of recruits, so we counted the recruits in the plots under a stereomicroscope before the transplant, and left them in a tank with running seawater overnight. The experiment was assembled in the following day.

We photographed each plot to monitor the survival of recruits after 2, 4, 8, 32 and 70 days.

To evaluate if predation could be the cause of differential mortality, we sampled the abundance of predator gastropods in both shores. Sampling was carried out in ten randomized blocks, with the factor Height (upper and lower midlittoral). We randomly selected 10 points where we put plots (20x20cm) in each zone of each shore, totalizing 40 plots. The sampling was carried out in the mid to late 2014.

Data analysis

Effects of biofilms on barnacle recruitment

We analyzed the NDVI values with a Mixed-Model ANOVA, with the factor Height on the shore (fixed, two levels) and Blocks (random, 5 levels). As the photo processing presented problems in the first samples, we eliminated the Factor time (7 levels) and carried out the analysis using the final data (200 days).

As barnacle recruitment was insignificant during all the experiment (see results), we could not analyze the effects of Height and Biofilm on density of recruits.

Differential barnacle mortality

We analyzed the variation in the proportion of living barnacles plots with a Mixed-Model ANOVA, with three main effects, Shore (fixed, two levels), Height on the shore (fixed, two levels), and Blocks nested in Shore (random, 7 levels). We transformed the data to arc sine of square root to obtain homoscedasticity, following Underwood (1997). Homoscedasticity was evaluated with Cochran's test.

We analyzed the density of predators with a Mixed-Model ANOVA, with the factors Height on the shore (fixed, two levels) and Blocks (random, 10 levels). Shores were analyzed separately to evaluate if patterns were similar.

The significance level used in all analysis was $P < 0.05$.

Results

Effects of biofilms on barnacle recruitment

Barnacle recruitment during the whole exposure time was insignificant in both heights (upper and lower midlittoral), being restricted to a few individuals in two plots. One plot had just a few recruits of the barnacle *Chthamalus bisinuatus* in the upper midlittoral, whereas the other had a few recruits of the barnacle *Tetraclita stalactifera* in the low midlittoral. Thus, due to the lack of barnacle recruitment, the experiment could not be concluded.

We analyzed differences in biofilm according to height on the shore in the 30 plots sampled after 200 days of exposition (example in Figure 5. This analysis showed that the biofilm is more abundant in the lower midlittoral (mean 0.21 ± 0.015 standard error) than in the upper midlittoral (mean 0.34 ± 0.02 standard error), since the NDVI values are significant higher in this zone (Table VI).

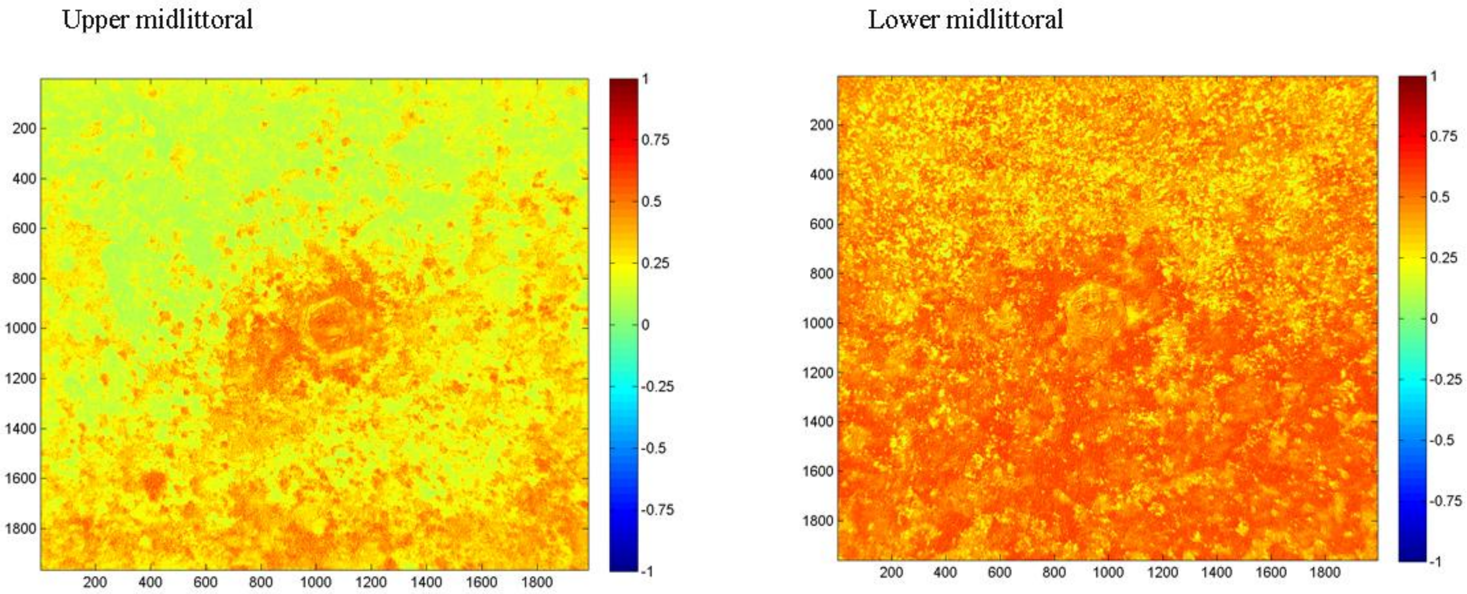

Figure 5. NDVI values of upper and lower midlittoral plots after 200 days of exposure.

Table VI. Mixed-Model ANOVA results comparing the NDVI in the upper and lower midlittoral of sheltered rocky shores. * $P < 0.05$.

Source of variation	df	MS	F
Block	4	0.005	1.25
Level	1	0.038	9.43*
Error	4	0.004	

Differential barnacle mortality

Barnacle mortality rate was higher in Lamberto than in Enseada, and higher in the lower midlittoral than in the upper midlittoral at the end of the experiment (Table VII).

Table VII. Mixed-model ANOVA comparing barnacle mortality in the upper and lower midlittoral of sheltered rocky shores after 70 days. ** $P < 0.01$

Source	df	MS	F
Shore	1	0.170	1.15
Height	1	2.474	10.29**
Height*Shore	1	0.159	0.66
Block(Shore)	12	0.147	0.61
Error	12	0.241	

By the end of the experiment, average values of barnacle density in the upper midlittoral plots was almost the half initial barnacle densities in both shores; in the lower midlittoral, only 20% of initial values were recorded in Enseada, whereas no survivals were recorded in Lamberto (Figure 6).

In the upper midlittoral, 50% of the population died after 70 days in both shores, whereas in the lower midlittoral, 50% of the population died in just about 10 days in Lamberto and 20 days in Enseada.

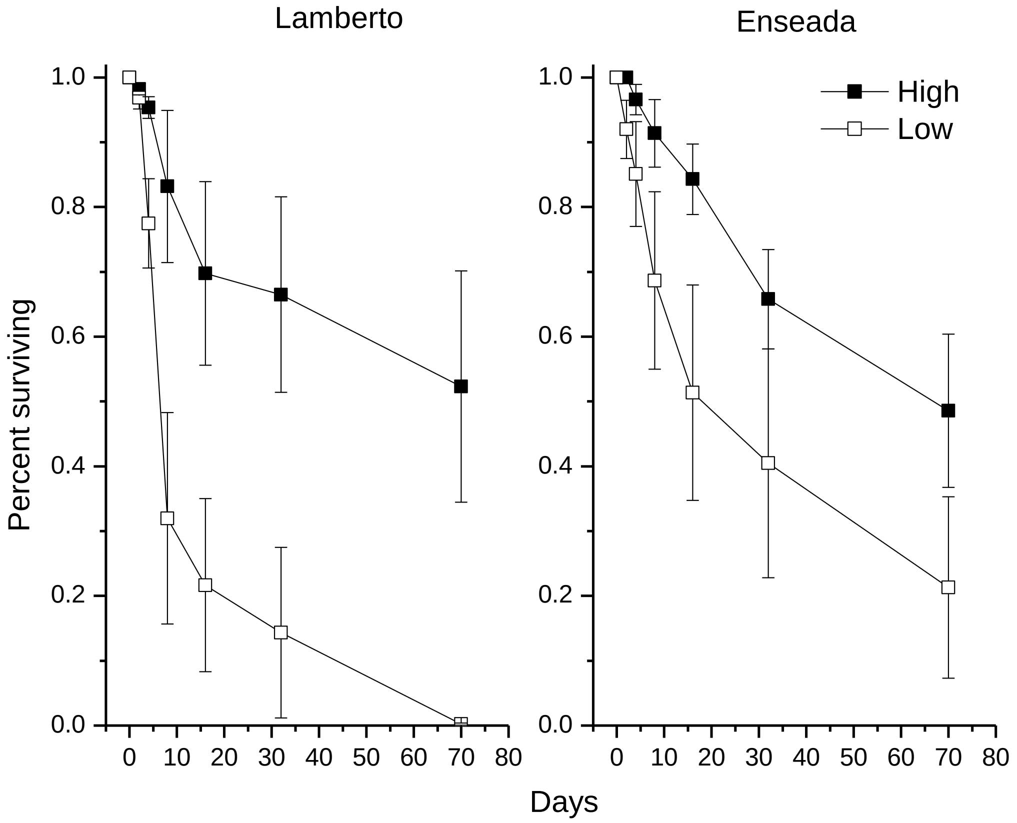

Figure 6. Survival of the barnacle *Chthamalus bisinuatus* in the upper and lower midlittoral of Lamberto and Enseada shores.

Sedentary predators found in the sheltered rocky shores studied were the gastropods *Stramonita haemastoma* and *Morula* sp. Both species were found in Lamberto, whereas in Enseada we found only *S. haemastoma*.

In a general way, these predators were not abundant in the plots, but all predators found were present in the low midlittoral. *S. haemastoma* occurred in 10% of the plots in Lamberto and in 30% of Enseada. *Morula* sp. was even less abundant, occurring in 20% of the plots in Lamberto. Consequently, we did not found significant differences in the abundance of predators between heights, but there was a trend to predators to occur in higher density in the low midlittoral of both shores ($P = 0{,}081$; Table VIII).

Table VIII. Mixed-Effects ANOVA comparing the predator occurrence in the upper and lower midlittoral in the sheltered rocky shores studied.

	Lamberto			*Enseada*	
Source	df	MS	F	MS	F
Block	9	0.117	1.0	0.117	1.0
Level	1	0.450	3.85	0.450	3.87
Error	9	0.117		0.117	

Discussion

Barnacles have been extensively used as models to study ecological processes, since they have a widespread distribution in different regions and have diverse roles in the benthic community of rocky shores (Bertness 1989, Jenkins & Hawkins 2003, Thomsen et al. 2010). In the present study, barnacle populations of *Chthamalus bisinuatus* are directly correlated with a zonation pattern, characterizing the midlittoral of Southeastern Brazil sheltered rocky shores.

Barnacle larvae can present different settlement responses to biofilm characteristics (Thompson et al. 1998, Lau et al. 2005), because they may have a selective behavior to biogenic substrates (Hug et al. 2007). Thus, considering that biofilm occurred in higher abundances in lower parts of the midlittoral on the sheltered rocky shores studied, and that we could not evaluate barnacle settlement in these zones precisely, the hypothesis that the biofilm influences the zonation patterns cannot be discarded. Oliver et al. (2000) found that biofilms developing in areas with higher times of immersion, as in the case of the lower midlittoral studied here, present a higher density of bacteria, and this might cause cypris inhibition.

The use of NDVI to measure the amount of chlorophyll is a precise and not destructive method (Murphy et al. 2006). However it allows evaluating just the photosynthetic part of the complex, being necessary the combined use of other techniques to evaluate the amount of heterotrophic components. In this study, we used the NDVI as a measure of biofilm abundance, and we found that the biofilm presents a differential pattern of abundance, being more abundant in lower midlittoral. As biofilm can concentrate environmental substances through absorption, and as these substances can have negative influences on barnacle recruitment by chemical signaling, differences in biofilm densities could influence recruitment patterns (Wieczorek et al. 1996, Henschel & Cook 1990).

Our results showed that barnacle recruitment was low in the sheltered rocky shores studied and that this result was not dependent on the zones or rock heights (upper and low midlittoral). In this way, the zonation pattern studied, which consists in almost absence of barnacles in the intermediate and lower midlittoral, could be influenced by periods with general low recruitment, being not necessarily related with a differential recruitment (with fewer recruits in lower zones). Zonation patterns might not be correlated with differential recruitment where biological post-settlement interactions have more influence (Connell 1970, Benedetti-Cecchi et al. 2000). However, we could not eliminate the possibility that the low recruitment during the experimental time resulted from an atypical year in barnacle reproduction or larval survival. Thus, the results found might not represent a long-lasting scenario of community structuration.

The method used did not allow a direct verification of settlement on the plots, only recruitment, since the newly attached larvae could not be visualized by naked eye or conventional digital photographs. Therefore, it is possible that differential settlement occurs in these shores, being higher in the upper zone. In this case, the metamorphosed larvae did not persist, since the period just after barnacle settlement might consist in a bottleneck for post-larval survival (Gosselin & Qian 1996).

Early barnacle post-settlement mortality is related with local conditions, such as coastal morphology, wave action and biological disturbances (Connell 1985, Hunt & Scheibling 1997). Just after the settlement, the earlier juvenile barnacles are also more vulnerable to environmental stress, as caused by changes in temperature and desiccation potential (Foster 1971). These factors could have influenced our results, since the summer, autumn and winter of 2014, when the experiments were carried out, were atypical. According to climatological bulletins of São Paulo University to the period, temperatures in São Paulo were higher than climatological averages (1933-2013; increase of 1ºC in autumn and winter and 3ºC in summer), as well as precipitation, which was lower than average both in summer (almost 50% of mean) and winter (about 75% of mean). As barnacles usually live close to lethal temperatures in their environment (Foster 1969), temperature increases could affect their populations (Chan et al. 2006).

Even though vulnerability tends to decrease with increases in individual sizes (Werner & Gilliam 1984), it is important to study the mortality processes of juvenile and adult phases as well, understanding the population dynamics in a holistic way. Our barnacle mortality results were more conclusive, since we could identify differential mortality to barnacle recruits according to the height on the shore. Mortality factors along barnacle life cycle include mostly smothering competition, both intra- (Bertness 1989) and inter-specific (Denley & Underwood 1979) or predation (Paine 1971) interactions.

In the sheltered rocky shores studied, the midlittoral was not occupied by any other large sessile organisms. Thus, barnacle mortality might be explained by predation by mobile consumers. Although predator abundances did not differ between zones, all predators found were present in the lower midlittoral. Fairweather & Underwood (1991) found that predation effects are reflected at small spatial scales, and some studies found that barnacle predation could be higher in lower zones, determining zonation patterns (Connell 1970, Delany et al. 2003). Predator abundances in the studied shores did not justify the higher mortality rate at Lamberto when compared to Enseada, so predator exclusion experiments are necessary to test this hypothesis.

An investigation on zonation patterns between the barnacles *Chthamalus montagui* and *Chthmalus stellatus* suggests that *C. montagui* displays higher mortality rates in lower shore levels due to longer periods of submersion (Delany 2003). Thus, the longer immersion in the lower midlittoral is other possible cause to explain the higher mortality of *C. bisinuatus* in this zone than in the upper zone of the sheltered rocky shores studied. Even though, this hypothesis does not explain the differences between shores.

The present study contributes to evaluate processes structuring the benthic communities of Brazilian sheltered rocky shores. Although we could not conclude the experiment of biofilm influence, the differential distribution of biofilm between heights suggests that this interaction can influence barnacle distributions. Also, the zonation pattern found can be the result of combined factors, with significant influence of barnacle differential mortality in the lower midlittoral.

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Considerações finais

Relativamente poucas espécies podem ser responsáveis pela caracterização de zonas em costões rochosos, como o encontrado para o mesolitoral de costões subtropicais neste estudo. Essa zona foi caracterizada pelas cracas *Chthamalus bisinuatus* e *Tetraclita stalactifera,* mexilhões (*Brachidontes* spp.), ostras (*Crassostrea* sp.), vermetídeos (*Petaloconchus* sp.), litorinídeos (*Nodilittorina lineolata*), macroalgas articuladas e incrustantes, e lapas (incluindo *Collisella subrugosa* e *Siphonaria hispida*). De modo geral, os organismos apresentaram variação espacial vertical, uma vez que houve variação de ocorrência dentro das três subzonas do mesolitoral. A variação espacial horizontal esteve associada a diferenças na abundância dos grupos entre costões, apesar de grande parte da variância ocorrer dentro de costões.

Os padrões de distribuição de espécies na comunidade bentônica foram condizentes com demais estudos que revelam pequenas escalas espaciais de variação, mas alguns táxons podem apresentar padrões distintos. Neste estudo, uma maior escala de variação ocorreu para as cracas *C. bisinuatus* e *T. stalactifera*, sendo que a abundância de *C. bisinuatus* foi maior na região de São Sebastião do que em Ubatuba e a ocorrência de *T. stalactifera* se deu em faixas mais largas em Ubatuba do que em São Sebastião, assim como em 2013 do que em 2014. Além disso, a disponibilidade de rocha nua, que foi abundante em todo o mesolitoral, também variou regionalmente em 2013. Hipóteses que explicariam a existência desses padrões de variação incluiriam a atuação da predação ou de fatores abióticos como alterações na hidrodinâmica (espacialmente) e na temperatura (temporalmente).

A pequena escala de variação espacial para as comunidades bentônicas de costões rochosos é usualmente associada à complexidade desses ambientes, marcados pela ocorrência de gradientes ambientais em pequenas distâncias espaciais e pela atuação de diversas interações biológicas. Neste sentido, os costões rochosos abrigados estudados podem ser considerados como ambientes complexos, com uma possível combinação de fatores atuando na determinação do padrão de zonação dentro do mesolitoral.

Os experimentos realizados neste estudo foram fundamentais para uma avaliação mais precisa dos fatores com influência no padrão analisado, apesar de não ter sido possível avaliar diretamente a influência do biofilme no recrutamento de *C. bisinuatus*. Como o recrutamento de cracas foi muito baixo durante todo o estudo, este resultado indica que as comunidades analisadas poderiam ser estruturadas por recrutamento. Nesse caso, a baixa abundância de cracas seria uma consequência de longos períodos sem o estabelecimento de quantidade significativa de recrutas.

O papel de biofilmes no recrutamento de macro invertebrados tem sido avaliado em diversos trabalhos, mas com diferentes resultados, o que evidencia forte dinâmica na interação. Como a análise da abundância de biofilme mostrou que os microorganismos fotossintetizantes são mais abundantes no mesolitoral inferior do que no superior, não podemos descartar a hipótese de haveria um efeito do biofilme no assentamento de cracas nos ambientes estudados. No caso, o padrão de zonação seria resultante de uma soma de fatores, já que indivíduos recém-assentados poderiam ter sofrido rápida mortalidade e já que o experimento com recrutas demonstrou maior mortalidade de *C. bisinuatus* nas zonas mais inferiores do mesolitoral.

As taxas de mortalidade considerando as duas zonas sugerem forte efeito desse fator na determinação do padrão de zonação. Ao término do experimento de mortalidade, os valores médios para a sobrevivência de *C. bisinuatus* no mesolitoral superior foi cerca da metade do número inicial de organismos, enquanto no mesolitoral inferior esse valor chegou à zero em uma das praias. Padrões de mortalidade diferencial para populações de cracas foram encontrados em alguns estudos e geralmente foram influenciados por predação. Apesar de não termos amostrado muitos predadores na área estudada, todos os indivíduos encontrados estavam na região inferior do mesolitoral, o que sustenta essa hipótese. Outra possibilidade inclui o maior tempo de imersão nas zonas baixas como causa da maior mortalidade de cracas. No entanto, asserções mais seguras só poderiam ser realizadas com a realização de um experimento de exclusão de predadores.

Ainda quanto à metodologia, observamos que a análise de variação espacial, a qual fez uso de transectos verticais, foi adequada para testar a variação da comunidade nas três escalas espaciais propostas, evitando qualquer tendência. Entretanto, limites mais bem definidos entre as subzonas poderiam ter sido atingidos em um estudo com amostragem horizontal dessas faixas, uma vez que as parcelas seriam posicionadas diretamente nas alturas de interesse

Consideramos que os resultados atingidos com a amostragem da variação espacial e com as investigações experimentais foram complementares. Assim, eles puderam colaborar com o entendimento da estruturação das comunidades de costões rochosos abrigados subtropicais, gerando um quadro de referência sobre os ambientes estudados.