Research Article

Comparative study of the estimated sample size for benthic intertidal species and communities

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ABSTRACT. The objective of this study was to determine the minimum sample size for studies of community structure and/or dominant species at different heights of a rocky intertidal zone at Rio de Janeiro. Community structure indicators suggested a variation in the minimum surface of 100 to 800 cm^2, with a minimum of 2 to 8 profiles and at least 20 to 80 quadrant sampling points, depending on the height. Indicators of species abundance suggest 100 cm^2 for *Hypnea musciformis* and 400 cm^2 for *Ulva fasciata*, *Phragmatopoma lapidosa* Kinberg, (1867) and *Gymnogongrus griffthsiae* at lower heights; 200 cm^2 for *Chthamalus* spp. at intermediate heights; and 800 cm^2 for *Littorina ziczac* at the greatest height. In general, seven to eight profiles and 10 to 20 sampling points were used. Different sample sizes were related to the abundance and spatial distributions of individual species, which varied at each intertidal height according to the degree of environmental stress.

Keywords: sample size, benthic community, intertidal, breakwater, northern Rio de Janeiro, Brazil.

Estudio comparativo del tamaño estimativo de muestra para especies bentónicas intermareales y de la comunidad

RESUMEN. El objetivo de este estudio fue determinar el tamaño mínimo de muestra para estudios de estructura de la comunidad y para las especies dominantes a diferentes alturas, en una zona intermareal rocosa en Río de Janeiro. Los indicadores de la estructura de la comunidad sugirieron una variación en la superficie mínima de 100 a 800 cm^2, 2 a 8 el número mínimo de perfiles y 20 a 80 el número mínimo de puntos de muestreo de cuadrantes, dependiendo en la altura. Los indicadores de abundancia de especies sugieren 100 cm^2 para *Hypnea musciformis*, 400 cm^2 para *Ulva fasciata*, *Phragmatopoma lapidosa* Kinberg, (1867) y *Gymnogongrus griffthsiae* a las alturas inferiores; 200 cm^2 para *Chthamalus* spp. a las alturas médiales y 800 cm^2 para *Littorina ziczac* a la altura superior. El número de perfiles y puntos de muestreo fue, en general, 7-8 y 10-20, respectivamente. Diferentes tamaños de la muestra fueron relacionados con la abundancia de especies individuales y su distribución espacial, que varían en cada altura de la zona intermareal de acuerdo con el grado de stress ambiental.

Palabras clave: tamaño de muestra, comunidad bentónica, intermareal, rompeolas, norte de Río de Janeiro, Brasil.

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INTRODUCTION

Sample representation is one of the main issues in benthic community studies. It should consider two aspects of the community: specific and structural (Ballesteros, 1986) and should be large enough to take into account the sample size and number. According to Omena *et al.* (1995), a minimal number should match sampling effort with statistical requirements.

Methods to determine the minimum sample size include species/area (Weinberg, 1978; Boudouresque & Belsher, 1979), diversity/area (Ballesteros, 1986; Vives & Salicrú, 2005) and similarity/area curves (Weinberg, 1978; Ballesteros, 1986; Murray *et al.*,

2006), with the stabilization of the selected criteria to determine the appropriate sample size. Ballesteros (1986) states that the criteria for defining the minimum area is always subjective and according to Hawkins & Hartnoll (1980), the best solution is one in which each investigator establishes his own. Boudouresque & Belsher (1979) propose the Molinier point x/y to determine the minimum area, which corresponds to the species/area curve where the increase of x% over the area corresponds to the increase of y% in the number of species.

The minimum sample size can also be determined for each species separately and several formulas were proposed, which consider i) the standard error of the average species abundance (Andrew & Mapstone, 1987; Kingsford & Battershill, 1998 in Murray *et al.*, 2006), ii) the standard deviation and the desired precision level as a proportion of the species abundance (Andrew & Mapstone, 1987; Murray *et al.*, 2006) and iii) an allowable error considering the confidence limits of 95% (Snedecor & Cochran, 1989; Murray *et al.*, 2006).

In Brazil, Rosso (1995) presented a theoretical approach about this issue and proposed the determination of sample size by the application of statistical models. Omena *et al.* (1995) determined the optimal sample number for the fouling community in Guanabara Bay, Rio de Janeiro, through panels. Experimental studies on consolidated substrates along the Brazilian coast generally refer to zonation (Oliveira-Filho & Mayal, 1976; Masi *et al.*, 2009a), succession (Breves-Ramos *et al.*, 2005), competition (Sauer-Machado *et al.*, 1996), herbivory (Apolinário *et al.*, 1999), phytosociology (Villaça *et al.*, 2008) or study methods (Sabino & Villaça, 1999; Moysés *et al.*, 2007), without a prior determination of the sample size.

Since different benthic groups are associated on a narrow intertidal zone it is hypothesized that the sample size for studies of the respective community structure and/or the abundant species should vary in this small spatial scale (centimeters). In this study we intend to answer the question about the minimum sample size needed in different experimental designs (quadrat size, number of profiles and number of points in the quadrat) of the intertidal benthic community in a rocky coast off Rio de Janeiro, combining feasible logistics and a robust mathematical/statistical point of view.

MATERIALS AND METHODS

Study area

The study was conducted in a breakwater with a set of granitic blocks, located on Farol de São Tomé beach (22°02'S, 41°03'W), northern coast of the State of Rio de Janeiro (Fig. 1). The rocky substrate is 10 m away from the beach; it is approximately 30 m in length and has a total inclination of 50º. Tidal regime is semidiurnal and amplitude is about 1.5 m.

Sampling design

The sampling process was realized between April and June 2007 during low tide always on smooth surfaces relatively perpendicular with its external faces facing the sea. We looked for rocky slope with higher similarity but as a breakwater it was not always possible mainly due to its extension (~30 m). Due to the substrate discontinuity, the determination of each height was performed by section with similar slope when possible (Gevertz, 1995). A total of six height levels compose the intertidal region of the rocky substrate: height 1 (0.2 to 0.6 m of tide level), height 2 (0.6 - 1.0 m), height 3 (1.0 - 1.4 m), height 4 (1.4 - 1.8 m), height 5 (1.8 - 2.2 m) and height 6 (2.2 - 2.6 m). In this case, heights are related to the distance on shore from low water. The main representative species in each level is: *Hypnea musciformis* (Wulfen) Lamouroux and *Ulva fasciata* Delile 1813 (height 1), *Phragmatopoma lapidosa* Kinberg, (1867), (Kröyer, 1856) and *U. fasciata* (height 2), *Gymnogongrus griffithsiae* (Turner) Martius and *Chthamalus* spp. (height 3), *Chthamalus* spp. (heights 4 and 5) and *Littorina ziczac* (Gmelin, 1791) (height 6).

Sampling was performed by photo-quadrat technique. Eight vertical profiles with a 2 m distance were sampled from 0.2 m above tide level to a selected point above the organism on the highest portion of the rocky substrate. Along each profile the quadrats were photographed next to a PVC structure of 1600 cm^2. Each photograph was analyzed for percent cover using the CPCe V3.4 (Coral Point Count for Excel) software program, which estimates bare space and the species percentage cover applied to a digital grid of points in the photograph. The distinction between primary and secondary canopy was not considered.

Data analysis

The minimum quadrat size was evaluated at each intertidal height level in eight sample profiles by analyzing quadrats with 100, 200, 400, 800 and 1600 cm^2 with 100 intersection points. Upon obtaining the minimum quadrat size, 1, 2, 4 and 8 profiles were randomly selected and analyzed, also considering 100 points of intersection in each quadrat. The minimum number of points in the quadrat was determined after obtaining the area and the number of profiles. To this end, we analyzed 10, 20, 40 and 80 points randomly
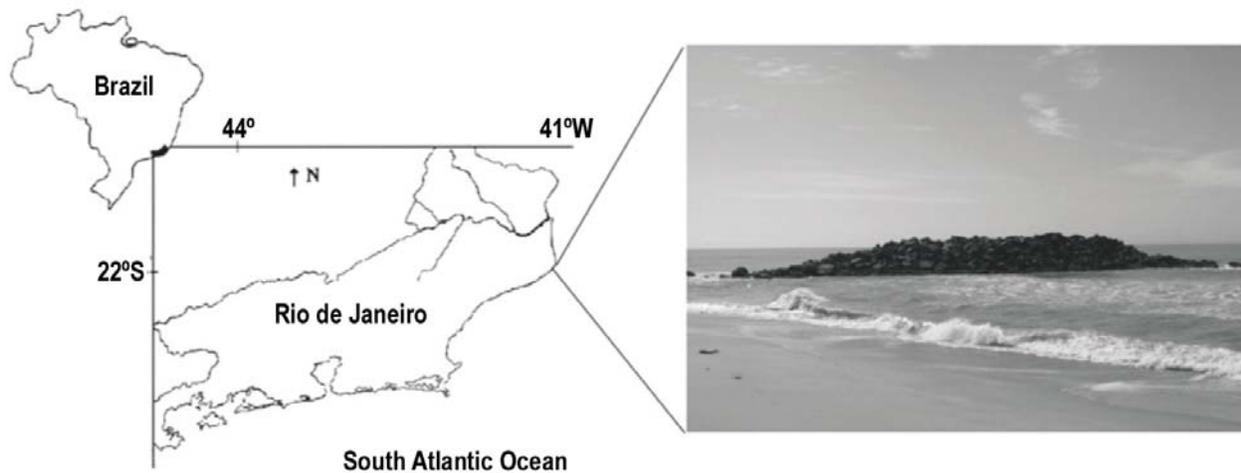
Figure 1. Studied area at Farol de São Tomé beach, northern coast of Rio de Janeiro.
Figura 1. Área de estudio en la playa del Farol de São Tomé, costa norte de Rio de Janeiro.

selected in a grid of 100 points, each corresponding to 100% percent coverage.

Comparative analysis

Comparative analysis of minimum sample size through indicators of community structure in the intertidal zone included: 1) the Molinier point 100/20 (Molinier, 1963 in Boudouresque & Belsher, 1979), where an increase of 100% for each sample size corresponds to an increase of 20% on species richness and Brillouin diversity (H, $\log_e$); 2) the accumulated species richness, considering the inclusion of 90% of the species (according to Murray *et al.*, 2006); 3) the community similarity in contiguous sample sizes (100 x 200 cm^2, 200 x 400 cm^2, 400 x 800 cm^2, 800 x 1600 cm^2; 2 x 4 profiles, 4 x 8 profiles and 10 x 20 points, 20 x 40 points, 40 x 80 points) with the Bray-Curtis index, considering the minimum sample size starting at 80% similarity.

Comparative analysis of minimum sample size through indicators of the representative species (> 10% of average coverage) included: 1) Molinier point 100/20; 2) increasing sample sizes (area, profile and number of points) versus SE/x, where SE = standard error and x = average species abundance (Kingsford & Battershill, 1998 in Murray *et al.*, 2006), with the criteria being the decrease and/or the stabilization of the curve; 3) increasing number of profiles *versus* $[SD/px]^2$, where SD = standard deviation, *P* = desired accuracy as a proportion of the average species abundance (= x) (Andrew & Mapstone, 1987; Murray *et al.*, 2006), with the criteria related on the adopted repeatability; 4) increasing number of profiles *versus* $4s^2/L^2$, where s^2 = variance in average species abundance and L = acceptable error for a 95%

confidence interval (Snedecor & Cochran, 1989; Murray *et al.*, 2006), with the criteria related on the accepted angular error.

RESULTS

Indicators of community structure

The determination of the minimum quadrat area, number of profiles and quadrat points for community studies through the Molinier point for species richness and diversity, the accumulated species number and the community similarity varied markedly on the six investigated height levels (Table 1). Based on all the above criteria and choosing the largest size among them for the determination of minimum quadrat size, we obtained 800 cm^2 at heights 1 and 6, 200 cm^2 at heights 2 and 4, 400 cm^2 at height 3 and 100 cm^2 at height 5 (Table 1). These were used to determine the number of profiles and points. Considering the number of profiles and choosing the largest size among them to determine the minimum number we obtained: 4 profiles at height 1, 8 profiles at heights 2 to 5 and 2 profiles at height 6 (Table 1). Consequently, these were used to determine the number of sampling points in the quadrats. Based on the criteria used above and choosing the largest size among them to determine the minimum number of points in the quadrat, we obtained: 80 points at heights 1, 3 and 6; 20 points at heights 2 and 4 and 40 points at height 5 (Table 1).

Indicators of individual species abundance

Quadrat size. The Molinier point for the relative abundance of the representative species at height 1 corresponded to 100 cm^2 for *Hypnea musciformis*

Table 1. Sample size required to quadrat size, number of profiles and number of points in the quadrat considering the Molinier point (Mol.) 100/20 criteria for species richness (R) and diversity (D), accumulated richness (AR) and similarity assemblages (SI) at six height levels of the intertidal rocky investigated. The suggested minimum sizes (S) are in bold.

Tabla 1. Tamaño necesario para el área del cuadrado de cada muestra, número de perfiles y número de puntos en el cuadrante considerando el criterio de punto de Molinier (Mol.) 100/20 para riqueza de especies (R) y diversidad (D), riqueza acumulada (AR) y similaridad (SI) de la comunidad en las seis alturas de una zona intermareal rocosa. Los tamaños mínimos sugeridos (S) están en negrita.

Height levels	Quadrat size (cm^2)					Number of profiles					Number of points				
	Mol.		AR	SI	S	Mol.		AR	SI	S	Mol.		AR	SI	S
	R	D				R	D				R	D			
6 (2.2 - 2.6 m)	100	100	100	800	**800**	2	2	2	2	**2**	80	80	80	10	**80**
5 (1.8 - 2.2 m)	100	100	100	100	**100**	2	4	8	2	**8**	10	10	40	10	**40**
4 (1.4 - 1.8 m)	100	100	200	100	**200**	1	4	8	1	**8**	20	10	10	10	**20**
3 (1.0 - 1.4 m)	400	400	400	100	**400**	1	1	8	4	**8**	40	20	80	10	**80**
2 (0.6 - 1.0 m)	200	200	200	100	**200**	2	2	8	1	**8**	20	10	10	10	**20**
1 (0.2 - 0.6 m)	800	800	100	100	**800**	1	1	4	4	**4**	40	40	80	10	**80**

(Wulfen) Lamouroux and 400 cm^2 for *Ulva fasciata* Delile, 1813; at height 2 at 100 cm^2 for *Phragmatopoma lapidosa* Kinberg (1867), (Krøyer, 1856) and 200 cm^2 for *U. fasciata*; at height 3 at 100 cm^2 for *P. caudata*, 200 cm^2 for *Gymnogongrus griffithsiae* (Turner) Martius and 800 cm^2 for *Chthamalus* spp.; at heights 4, 5 and 6 at 100 cm^2 for *Chthamalus* spp. and *Littorina ziczac* (Gmelin, 1791) (Table 2).

Plotting the standard error as a proportion of the average abundance *versus* sampled area revealed a function that tended to decline or to stabilize (Fig. 2). Based on the criteria above and choosing the largest size among them to determine the sampling area, we obtained: height 1-100 cm^2 for *H. musciformis* and 400 cm^2 for *U. fasciata*; height 2-400 cm^2 for *U. fasciata* and *P. caudata*; height 3-400 cm^2 for *P. caudata* and *G. griffithsiae* and 800 cm^2 for *Chthamalus* spp.; heights 4 and 5-200 cm^2 for *Chthamalus* spp., height 6-800 cm^2 for *L. ziczac* (Fig. 2, Table 2).

Number of profiles

The Molinier point for the relative abundance of the representative species at height 1 corresponded to 4 profiles for *Hypnea musciformis* and 8 profiles for *Ulva fasciata*; at heights 2, 3 and 4 corresponded to 1 profile for all species and at heights 5 and 6 corresponded to 2 profiles for *Chthamalus* spp. and *Littorina ziczac* (Table 2).

A graphical representation of the number of profiles versus the standard error as a proportion of the average species abundance showed a declining function, where the addition of more profiles reflected in a declining standard error, followed by a trend towards the stability of the curves, mostly from 4 profiles for all representative species at heights 1, 2, 3 and 5. Only for *Chthamalus* spp. at heights 3 and 4, a decreasing function was recorded with 8 sample profiles. *L. ziczac* at height 6 showed a tendency for stability of the respective curve starting at 2 profiles (Fig. 3a).

Plotting the number of profiles versus the desired accuracy level as a proportion of average species abundance in accordance with the formula $[DP/p\ x]^2$, showed that a greater precision (0.05) was directly related to a high number of profiles, between 33 and 1416. Assuming a precision of 0.15, we observed a sharp decrease but still above 8 profiles, except for *Phragmatopoma lapidosa* Kinberg, (1867) with 6 profiles at height 2 and *Chthamalus* spp. with 4 profiles at height 4. The minimum number of profiles proved to be equal to or lower than the largest sampled size (N = 8 profiles) with a precision value starting at 0.40 (Fig. 3b).

Plotting the number of profiles versus the variance on average species abundance for a confidence level of 95% with an allowed error of ± 2, a number of profiles superior to 170 is necessary at all heights and species, except for *L. ziczac* that required 4 profiles. With an allowed error of ± 8, there is a decrease in the minimum number of profiles, but it is still above 10. The minimum number of profiles proved to be equal
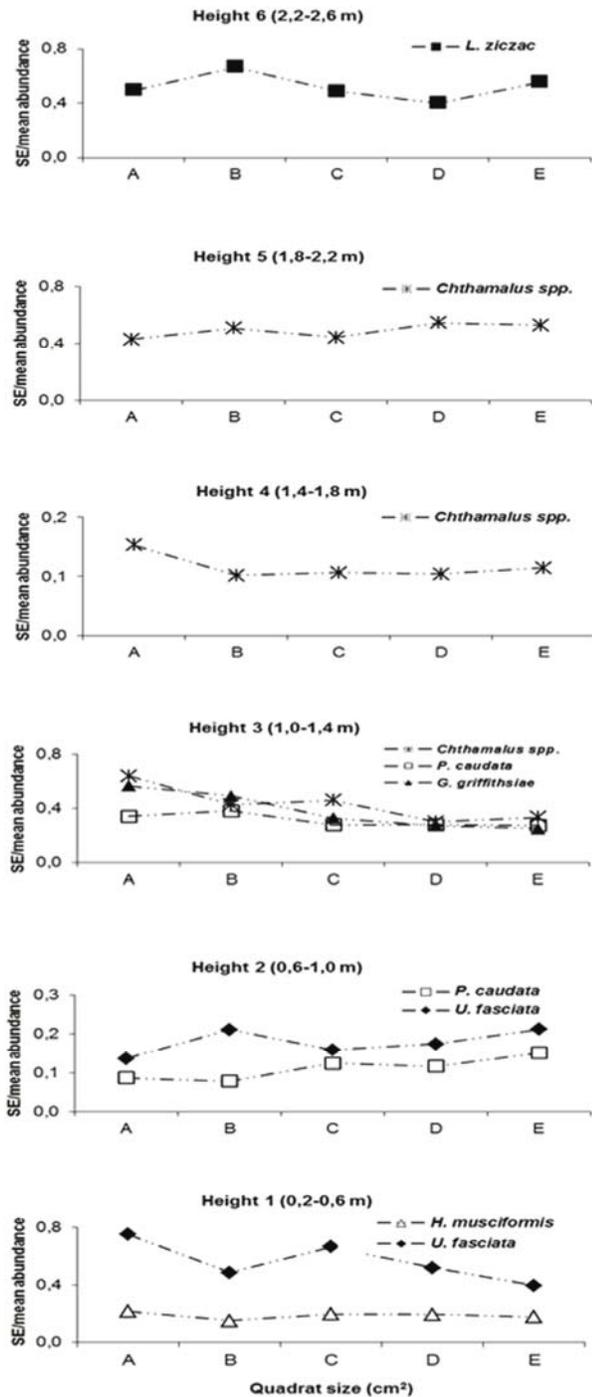
Figure 2. Standard error/mean abundance of the representative species on different sampled areas (A: 100 cm²; B: 200 cm²; C: 400 cm²; D: 800 cm²; E: 1600 cm²), at six height levels of the intertidal rocky zone (N = 8 profiles). Note the different y-axis scales.

Figura 2. Error standart/abundancia media de las especies más representativas en las diferentes áreas muestreadas (A: 100 cm²; B: 200 cm²; C: 400 cm²; D: 800 cm²; E: 1600 cm²), en las seis alturas da zona intermareal rocosa (N = 8 perfiles). Observe las diferentes escalas del eje y.

to or lower than the largest sampled size (N = 8 profiles) with an allowed error starting at ± 20 (Fig. 3c).

In summary, based on the criteria above investigated and choosing the largest size among them to determine the minimum number of profiles, we obtained more than 8 profiles for the representative species of the intertidal zone (Table 2).

Number of sampling points

The Molinier point for the relative abundance of the representative species corresponded to 10 points at heights 1 to 5, except for *Gymnogongrus griffithsiae* at height 3 and *Littorina ziczac* at height 6, which was achieved with a higher sample size, 40 points (Table 2). A graphical representation of the number of sampling points versus the standard error as a proportion of the average species abundance revealed stable values from the smallest number for almost all the species and heights except for *L. ziczac* at height 6 that required a maximum number of 80 points.

Based on the criteria above and considering the largest size among them to determine the minimum number of sampling points, we obtained at height 1: 10 points for *H. musciformis* and *U. fasciata*; height 2: 20 points for *U. fasciata* and *P. caudata*; height 3: 10 points for *Chthamalus* spp., 20 for *P. caudata* and 40 for *G. griffithsiae*; heights 4 and 5: 10 points for *Chthamalus* spp. and height 6: 80 for *L. ziczac* (Table 2).

DISCUSSION

Community structure

The Molinier point is considered as one of the oldest for determining the minimum sample size (Molinier, 1963 in Boudouresque & Belsher, 1979) and relates with the information content of a numerical descriptor. In this study, in each intertidal height level, the Molinier point corresponded to different sample sizes and in the lower shore the minimum sample area corresponded to 800 cm². Boudouresque & Belsher (1979) in a Mediterranean breakwater used only this criterion and obtained 100 cm² as the minimum area for benthic community in the lower shore.

Considering the accumulated richness, in which 90% of species were included, we agree with Ballesteros (1986) that in seeking the appropriate sample area it is necessary to obtain a representative fraction of its total richness. The whole community was not considered since this 10% difference represented a rare species (< 3% average coverage). Omena *et al.* (1995) also worked with the accumulated

Table 2. Sample size required to quadrat size, number of profiles and number of points in the quadrat considering the Molinier point 100/20 criteria and the formulas standard error/mean abundance (SE/x), $[DP/px]^2$ and $4s^2/L^2$ of the most representative species at six height levels of the intertidal rocky zone. The suggested minimum sizes (S) are in bold.

Tabla 2. Tamaño necesario para el área del cuadrado de cada muestra, número de perfiles y número de puntos en el cuadrante considerando el criterio de punto de Molinier 100/20 y las fórmulas error standart/abundancia media (SE/x), $[DP/px]^2$ y $4s^2/L^2$ de las especies representativas en las seis alturas da zona intermareal rocosa. Los tamaños mínimos sugeridos (S) están en negrita.

Height levels	Species	Quadrat size (cm²)			Number of profiles					Number of points		
		Mol	SE/x	**S**	Mol	SE/x	$[DP/p·x]^2$	$4s^2/L^2$	**S**	Mol	SE/x	**S**
6 (2.2 - 2.6 m)	*L. ziczac*	100	800	**800**	2	2	8	4	**8**	40	80	**80**
5 (1.8 - 2.2 m)	*Chthamalus* spp.	100	200	**200**	2	4	8	8	**8**	10	10	**10**
4 (1.4 - 1.8 m)	*Chthamalus* spp.	100	200	**200**	1	8	4	7	**8**	10	10	**10**
3 (1.0 - 1.4 m)	*Chthamalus* spp.	800	800	**800**	1	8	8	8	**8**	10	10	**10**
	P. caudata	100	400	**400**	1	4	7	8	**8**	10	20	**20**
	G. griffithsiae	200	400	**400**	1	4	7	7	**7**	40	10	**40**
2 (0.6 - 1.0 m)	*P. caudata*	100	400	**400**	1	4	6	8	**8**	10	20	**20**
	Ulva fasciata	200	400	**400**	1	4	5	8	**8**	10	20	**20**
1 (0.2 - 0.6 m)	*H. musciformis*	100	100	**100**	4	4	6	8	**8**	10	10	**10**
	U. fasciata	400	200	**400**	8	4	8	8	**8**	10	10	**10**

richness criterion, but they used the curve stabilization to determine the minimum sample number for the fouling community in Rio de Janeiro. In this study, we sought for less subjectivity. It was registered a high variability in species richness at small spatial scales (centimeters) along the vertical gradient in the studied breakwater and Masi *et al.* (2009b) attributed this to specific local environmental factors as air temperature, height and wave period together with air exposure to which the organisms are exposed.

Although a pilot study is recommended in each individual area, 400 to 500 cm² are considered as representative sizes for benthic communities throughout the intertidal region by many authors (Sabino & Villaça, 1999; Breves-Ramos *et al.*, 2005; Moysés *et al.*, 2007). In this study, we observed that different quadrat sizes are necessary for the intertidal community along its extension, which vary according to different environmental conditions. At heights 1 and 6, the minimum size corresponded to 800 cm², at height 3 was 400 cm² and in the others, even smaller areas were considered appropriate. At height 1 corresponding to the Infralittoral fringe (Masi *et al.*, 2009a), where environmental conditions are less stressful, the greatest richness and diversity values at 800 cm² quadrats were representative of the local community structure. At height 6 (Supralitoral Fringe

sensu, Masi *et al.*, 2009a), where environmental conditions are more stressful due to longer emersion periods, the greater variability in the abundance of the fewer species reflected in a low similarity between contiguous sample sizes, which was a determining factor of a higher minimum quadrat size.

Considering the number of profiles, the minimum size corresponded to the largest ones (N = 8 profiles) in most of the heights. The substrate consists of a breakwater with an accentuated degree of wave exposure. According to Bulleri & Chapman (2004), breakwaters are three-dimensional habitats and offer a variety of environments regarding orientation, shading and wave exposure. The high variability in species abundance between the profiles is attributed to the irregular topography of the substrate, providing different environmental conditions on a small scale, which favors the formation of different associations of species at the same height level (Masi & Zalmon, 2008). According to Murray *et al.* (2006), if the variability in species abundance is high, several samples are necessary, which was also observed in this study. It is worth emphasizing that the determination of the number of profiles is related to the area of the quadrat. According to Rosso (1995) adopting smaller and numerous elements helps to reveal the different microhabitats. A higher number of
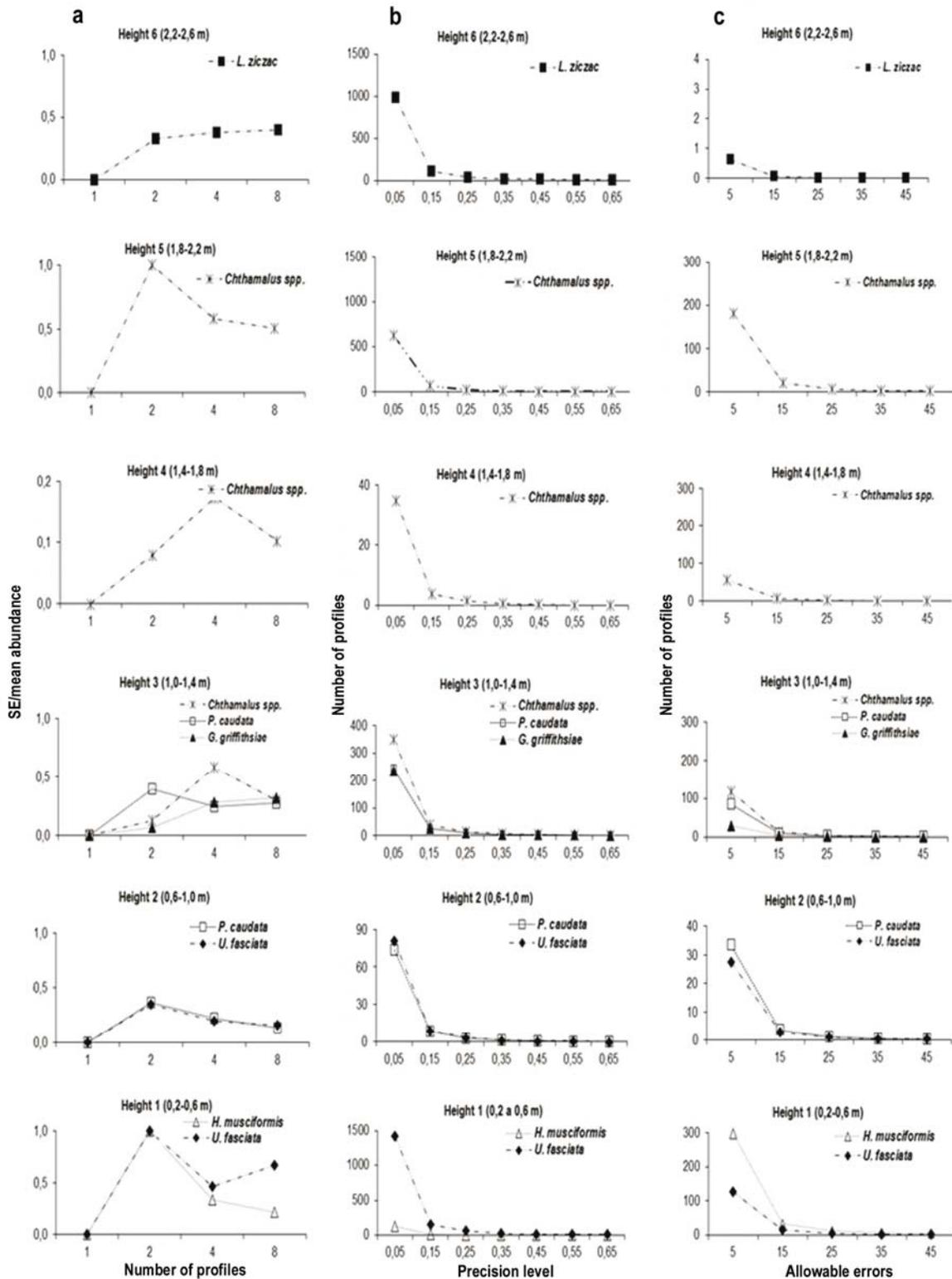
Figure 3. Number of profiles as a function of a) the standard error/mean abundance, b) the desired precision level in accordance with the formula $[DP/p \ x]^2$, c) the allowable errors in accordance with the formula $4s^2/L^2$ at six height levels of the intertidal rocky zone. Note the different y-axis scales.

Figura 3. Número de perfiles como función de: a) el error estándar /abundancia media, b) la precisión deseada de acuerdo con la fórmula $[DP/p \ x]^2$, c) los errores permitidos de acuerdo con la fórmula $4s^2/L^2$ en las seis alturas da zona intermareal rocosa. Observe las diferentes escalas del eje y.

profiles combined with smaller areas reflected in a detailed substrate sampling and showed the distribution of each organism. However a smaller number of profiles combined with larger quadrats can optimize the sampling time without interfering with the sample representation.

Regarding the number of sampling points, Sabino & Villaça (1999) found that few points in a quadrat produced a biased sampling, recording only the dominant species. In this study, the minimum size required was different along the intertidal zone: at heights 1, 3 and 6 corresponded to the maximum (N = 80). At heights 1 and 3, the presence of several rare species (> 40% of the total species with < 4% coverage average) with a patchy distribution probably contributed to a greater number of points. At height 6 the highest number of points also reflected the presence of few species with low abundance (± 5%) with a punctual distribution. The number of points was correlated with the sampled area, requiring higher numbers in larger areas.

Representative species

Sampling effort sometimes targets a specific component of the community so the knowledge of the relative abundance and its variation is crucial to determine the experimental design (Murray *et al.*, 2006). In the intertidal zone, the different environmental conditions along a narrow area influence the species distribution and abundance, reflecting different vertical assemblages. Thus, we chose to characterize the minimum sample size considering also the most representative species at different height levels of the studied intertidal substrate.

At height 1 where the air exposure time is lower, the most representative species *Hypnea musciformis* is an abundant macroalgae in the coast of Rio de Janeiro (Sauer-Machado *et al.*, 1996; Villaça *et al.*, 2008). The appropriate sampling size corresponded to the smallest quadrat and number of points. *Hypnea* showed a homogeneous distribution and covered about 100% in most quadrats, which may have contributed for smaller sizes. Rosso (1995) emphasized the importance of adopting a larger number of samples combined with smaller areas to study the distribution of each organism. *U. fasciata* was also characterized as representative of the Infralitoral fringe site. Sauer-Machado *et al.* (1996) registered *Ulva* sp. in lower intertidal levels, considering the Chlorophyta competitively inferior to *H. musciformis*. *U. fasciata* showed an average abundance of about 20% at height 1 and 35% at height 2, with the minimum quadrat size corresponding to 400 cm^2, 10-20 points and 8 profiles.

Its patchy distribution reflects a greater spatial variation on a small scale, which may result in larger sample size and in this case it will not underestimate the abundance of the species.

At heights 2 and 3, corresponding to lower Eulitoral (Masi *et al.*, 2009a), the most representative species *Phragmatopoma lapidosa* Kinberg, (1867) is a sandy reef constructer very common in this fringe (Masi & Zalmon, 2008; Masi *et al.*, 2009a). Sample sizes required at both height levels were 400 cm^2, 8 profiles and 20 points. In some quadrats, the polychaete formed an extensive reef covering the area and in others had a discontinuous dominance, and constitutes variable mosaics in the substrate. The topographic irregularity may have contributed to this distribution pattern and with the largest number of profiles.

Chthamalus spp., one of the most abundant at height 3 and dominant at heights 4 and 5 required 800 cm^2 at the lowest one and 200 cm^2 at the others. The minimum number of profiles and sampling points corresponded to 8 and to 10 points in all the heights above. Benedetti-Cecchi *et al.* (2000) observed the cirripede *C. stellatus* (Poli) with a scatter distribution in the lower level of the intertidal zone. In the present study *Chthamalus* spp. followed the same pattern, which may have influenced the adoption of larger sampling area at this height. At heights 4 and 5, the smallest area reflects the absolute dominance of the species, commonly recorded by several authors in the upper intertidal zone (Benedetti-Cecchi *et al.*, 2000; Coutinho & Zalmon, 2009).

At height 6, which corresponds to the Supralitoral Fringe (Masi *et al.*, 2009a), the representative species *Littorina ziczac* showed the necessity of the highest quadrat sizes (800 cm^2, 8 profiles and 80 points). According to Coutinho & Zalmon (2009), herbivorous gastropods are the most characteristic component in this fringe. In the study area the species was not abundant with a maximum abundance of 5%, although it is representative of this height by its resistance to air exposure and mobility. Judge *et al.* (2008) observed grazer gastropods occurring in the Supralitoral frequently clustered in crevices, which provide a milder condition and a reduced stress by desiccation. In this study, *L. ziczac* showed an irregular distribution on the substrate, which possibly explains the larger sample sizes for the species.

In summary, the required sample area showed a direct relationship with the abundance and spatial distribution of each species. Species with lower abundance and patchy distribution required larger sampling areas, while those more abundant and more evenly distributed corresponded to smaller areas.

According to Andrew & Mapstone (1987), the size concept is related to the aggregation scale of organisms. Benedetti-Cecchi et al. (1996) stated that the sample size might vary according to the study goal, the complexity of the habitat and the spatial distribution of the organisms. They found a high variation in the organism's distribution among their areas, also suggesting that the quadrat size was generally related to the scale of aggregation.

Regarding the number of profiles, a minimum of 8 was required for the most representative species of the substrate under study. The high number of profiles can be attributed to the wave exposure, which reflects species associations on a patchy distribution (Masi & Zalmon, 2008). Parravicini et al. (2009) affirm that a large number of replicates, 8 to 12, generally are adopted in studies using the photoquadrat method. We indicate a higher number of profiles than the maximum tested if greater accuracy and less error are required. Murray et al. (2006) found that the adoption of a smaller error using the formula $n = 4s^2/L^2$ resulted on a higher sample number. However, on the studied substrate the sampling effort on more than 8 profiles is logistically difficult due to the intense wave exposition and also to the extension of the breakwater (~30 m); they would be dependent sampling profiles.

Considering the number of points in the quadrat, it was observed that 10-20 points proved to be enough for the most representative species, with the exceptions of G. griffthsiae and L. ziczac, which are the rarest ones with irregular distributions, and reflected higher numbers than the others.

The influence of the temporal variability must be considered. In a recent study Masi et al. (2009b) reported on the same breakwater that the seasonal variation of the benthic community was restricted to a narrow intertidal band (1.0-1.2 m). So a temporal replicability on the sample size determination should be important at least on height 3.

The results show the importance of different sample sizes for quadrat area, number of profiles and sampling points at different height levels in the intertidal zone to study the community structure and each representative species. It is important to emphasize that the adoption of any criteria depends on the objectives and the logistical feasibility. The time-limited studies in the intertidal zone should also be considered without interfering with the accuracy and precision for the credibility of the results.

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