

Biodiversity monitoring in rocky shores: Challenges of devising a globally applicable and cost-effective protocol

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ABSTRACT

Large-scale coastal monitoring programs that focus on long-term inter-annual and seasonal community variability are rare mostly because they are costly, logistically complex and require coordination by groups of dedicated scientists. The Marine Biodiversity Observation Network (MBON) is currently developing a regional collaboration throughout the American continent to collect biological data in coastal habitats using common methodologies and sharing best practices. The goal of this paper is to compare two survey methods with contrasting field time demands (i.e. *in situ* collected data vs. photoquadrats), identify the scales at which they may differ, and determine the convenience and suitability for their use in large-scale standardized biodiversity studies on rocky intertidal shores. Visual quadrat (VQ) data collected in the field was compared with data obtained from photographs of those same quadrats (PQ) digitally analyzed at three intertidal levels within two sites during four different times of the year. Analysis by site showed a seasonal effect in all shore levels and an effect of methods only at the low intertidal level at both sites. The PQ method is a reliable, cheap and time efficient tool for the long-term study of rocky intertidal communities. It is capable of detecting the spatial and temporal variability as the VQ method at various scales including tidal height, time of the year and site. We suggest the use of the mid intertidal level as the standardized sampling zone across latitudes on the basis of having higher diversity than the high intertidal, more sampling time than the low intertidal level, and being more affected by climate change both through changes in the air (temperature and wind) and in the ocean (warming and acidification). This study provides empirical evidence that a simple, low-cost and low-tech method may offer the required information that large-scale monitoring programs need.

1. Introduction

Rocky shores are one of the most common coastal ecosystems globally (Emery and Kuhn 1982; Granja 2004). Unlike other types of structurally complex coastal ecosystems such as coral reefs, kelp forests, mangroves and salt marshes which have restrictive geographic ranges dependent on climatic and oceanic conditions, rocky shore ecosystems occur in tropical, subtropical, temperate and boreal environments (Emery and Kuhn 1982). Consequently, rocky shores have been a key

environment for ecological research for decades with studies focusing on patterns and processes from the local to the global scale (Paine 1994). For example, at a large scale, research have shown that functional and trophic structure can be similar across regions (Blanchette et al., 2009). At a regional scale, studies in different biogeographical regions have shown that considerable variability exists in the abundance of key open-coast taxa (Bustamante and Branch 1996; Broitman et al., 2001; Menge et al., 2004; Schoch et al., 2006).

Long-term, sustained, time-series of biodiversity, community

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structure and dynamics in this accessible ecosystem are not so common. Furthermore, existing data is affected by aspects such as the variability in the collection methods, heterogeneity in spatial and temporal sampling, and in data formats that make data incomparable (Duffy et al., 2019; Miloslavich et al., 2019). Monitoring efforts that try to capture both long-term inter-annual and seasonal community variability are even rarer, mostly because they are costly, logistically complex and require much coordination by different groups of dedicated scientists. The most spatiotemporally extensive time series for rocky intertidal systems is from the United Kingdom and France, the Marine Biodiversity and Climate Change Project (MarClim) which extends back to 1950 in over 100 survey sites. MarClim data has demonstrated some of the fastest shifts in species distributions in any natural system in response to climate change (Mieszkowska et al. 2006, 2007). In North America, the PISCO program on the US West Coast has been monitoring rocky intertidal sites since 1999 and has shown the contribution of climate cycles and climate change to ecological patterns on rocky shores (Menge et al., 2008; Iles et al., 2012). On the eastern Mediterranean coast, eight years of extensive, multi-site, annual and seasonal surveys have shown strong seasonal signals and inter-annual shifts in the abundance of key species, including a regional collapse of an abundant invader (Rilov et al., 2020a). In 2016, the Pole to Pole project of the Marine Biodiversity Observation Network (MBON P2P) was established as a regional collaboration throughout the American continent to assess marine biodiversity and ecosystem change using field and space observations. This project collects biological data in coastal habitats (rocky shores and sandy beaches) using common methodologies and sharing best practices (Canonico et al., 2019). It also contributes to measure relevant Essential Ocean Variables (EOVs) (e.g. 'macroalgal canopy cover and composition', and the emerging 'benthic invertebrate abundance and distribution', Miloslavich et al., 2018) as identified by the Global Ocean Observing System (GOOS) (Tanhua et al., 2019).

Along South American coasts there have been a few international programs in the last two decades that have made significant efforts in assessing the spatiotemporal variability in rocky shore biodiversity. Between 2005 and 2010, the Natural Geography in Shore Areas (NaGISA) project of the Census of Marine Life (CoML) program collected data in four countries with a standardized protocol contributing more than 7,000 records to the Ocean Biogeographic Information System (OBIS) (Miloslavich et al., 2013). Building on the NaGISA efforts, the South American Research Group on Coastal Ecosystems (SARCE) continued to assess and fill knowledge gaps on marine biodiversity and biomass between 2010 and 2013. In this period, the SARCE network sampled more than 150 intertidal rocky shore sites using a standardized protocol and covering a latitudinal range from 11° North to 55° South (Miloslavich et al., 2016a), contributing almost 20,000 records to the OBIS database.

Large-scale monitoring of intertidal habitats presents challenges that could limit their geographical extension and duration if not addressed (Hewitt et al., 2007). Sampling intertidal habitats has an intrinsic time constraint which is the actual time the substrate is exposed to air during low tide. Concurrently, biodiversity hotspot locations require large numbers of replicates to obtain representative data of assemblages. Hence, identifying and quantifying organisms using gridded quadrats in the field, within the short timeframe of a low tide, becomes time consuming and requires large amounts of personnel hours in the field. The NaGISA and SARCE projects used gridded visual quadrats to estimate percentage cover of organisms in the field (Rigby et al., 2007; Miloslavich et al. 2013, 2016a). During the SARCE sampling, in addition to visual counts, photos were taken of each quadrat, mainly as archive, but could potentially be used also for analysis. Thus, the goal of this paper is to compare the two survey methods (i.e. *in situ* collected data vs. photoquadrats) with contrasting field time demands, identify the scales at which they may differ, and determine the convenience and suitability for their use in standardized biodiversity studies on rocky intertidal shores.

2. Materials and methods

2.1. Study site

Sampling was carried out on the rocky intertidal shores of Punta Este (42° 47'S; 64° 57'W) and Punta Cuevas (42° 47'S; 65° 00'W). Sites are separated by about 10 km on the southwest coast of Golfo Nuevo, Chubut, Argentina. Sedimentary rock platforms (mudstone) are exposed to semidiurnal tides with mean amplitude of about 4 m. The characteristic three level biological zonation of Patagonian rocky intertidal shores was present at both sites: high (HT), mid (MT) and low (LT) tide. The HT has a large proportion of bare rock and common organisms were the invasive barnacle *Balanus glandula*, the pulmonate limpet *Siphonaria lessonii*, with the macroalgae *Ulva prolifera* and the crustose algae *Ralfsia* sp. seasonally present. The MT was completely dominated by a single layered matrix of scorched mussels composed of two species *Brachidontes rodriguezii* and *B. (Perumytilus) purpuratus*. The LT was characterized by several algal species such as *Codium* sp., *Ceramium* sp., *Dictyota dichotoma*, the invasive *Undaria pinnatifida* and a large proportion of the calcareous alga *Corallina officinalis* as well as the gastropods *Tegula patagonica* and *Trophon geversianus* (Bertness et al., 2006; Raffo et al., 2014; Miloslavich et al., 2016a).

2.2. Sampling

Data used in this study were collected during the SARCE program following a specifically designed protocol to study changes in rocky shore communities (SARCE 2012). Samples were collected in December 2013 and April, June and September 2014 during low tides. Percentage cover of sessile organisms was estimated using 50 × 50 cm quadrats haphazardly placed on the substrate. The visual quadrats (VQ) were gridded frames with 100 intersection points. Organisms observed underneath each intercept were identified and recorded to the lowest possible taxonomic level in the field. As part of the protocol, photographs of sampled plots were required as supporting material. Thus, a digital photograph of the same 50 × 50 cm plot (frame without grid) was taken and defined as a photoquadrat (PQ). In the lab, one hundred equidistant points were placed over the digital image and organisms observed under each point were determined to the lowest possible taxonomic level. PQs were analyzed using the free software Coral Point Count (CPCe V 4.1, Kohler and Gill 2006). Data from a total of 288 quadrats (144 VQ and 144 PQ) were used in this study (6 replicates, 3 levels, 2 sites and 4 seasons).

2.3. Data analysis

Non-metric multi-dimensional scaling (nMDS) was used to visualize multivariate patterns in benthic assemblages at various scales. Benthic assemblage data were analyzed separately for each site and shore level using permutational analysis of variance (PERMANOVA) with the PERMANOVA extension in Primer v6.1.7 software (Anderson et al., 2008). Similarity matrices based on Bray-Curtis measure were generated for the analyses, which used 999 permutations of residuals under a reduced model (Anderson et al., 2008). PERMANOVA model had two factors: Method (Me, fixed, 2 levels: VQ and PQ) and Season (Se, fixed, 4 levels: summer, autumn, winter and spring). Pairwise comparisons were performed among pairs of levels for significant factors to identify differences. To test for a correlation in percentage cover estimated by the two proposed methods, the RELATE routine (Spearman correlation) was used on similarity matrices of methods from each level and site (seasons were pooled). SIMPER analyses were used to determine which taxa contributed more to variation among samples between methods and levels. DistLM analyses were performed on low intertidal assemblage data to identify and visualize taxa that contributed most (>0.2 correlation) to the observed differences among methods, separately for each site.

3. Results

Overall, 24 sessile taxa were identified. Of these, 20 were shared by both methods and the four that were not shared were extremely infrequent and when present, had a very low cover (<3%). Visual analysis of assemblage data of all intertidal levels, sites, seasons and methods showed a clear separation of intertidal levels (Fig. 1A). Furthermore, analysis of each intertidal level showed a separation of samples by site in all levels being more noticeable in the MT and LT levels (Fig. 1B, C and D). No clear pattern of separation due to method was observed in any intertidal level (Fig. 1B, C and D). Analysis by site showed a seasonal effect in all shore levels and an effect of methods only at the LT level at both sites (Table 1). However, in the LT level differences between methods within each season were not observed through pair-wise comparisons at either site (Table 2a).

The similarity matrices based on percentage cover estimated by VQ and PQ were well correlated for the three intertidal levels at both sites (Table 2b). High ρ values were obtained for the LT and HT levels at both sites whilst the MT showed lower values (Table 2b). The SIMPER results show that the taxa that explain similarity among assemblages were generally the same for both methods (Table 2c) at all intertidal levels. The number of taxa needed to reach 90% of accumulated contribution was lowest for the HT and highest for the LT.

DistLM analyses on LT assemblages from both sites showed that taxa responsible for the observed patterns differed between sites (Fig. 2A and B). More than 70% of the total variation was explained by the two axes at both sites, yet no trend towards a separation associated to methods could be easily observed (Fig. 2A and B). Coralline algae was the only taxa that showed a high contribution to the explained variation at both sites (Fig. 2A and B).

4. Discussion

The selection of a method for large-scale monitoring should recognize the aspects that may limit their spatial and temporal extent (Hewitt et al., 2007). Programs that focus on intertidal habitats are compelled to

work between tides. The time demand of the selected method in combination with the available resources, both human and financial, determine the potential sampling area and/or replication that may be accomplished. Hence, finding a method that maximizes the use of resources, reduces impacts on the study habitat and obtains robust data is essential. In this study, we found a cost-effective method that can obtain reliable rocky shore biodiversity data. This simple photographic method can be suitable and can save much time in the field, especially where the communities are not too structurally complex or multilayered (e.g., with a thick algal canopy).

Sampling time is one of the main aspects that may define the duration and spatial breath of field work and it is directly related to costs. Much like the time limitations encountered when sampling rocky subtidal environments through SCUBA diving (Sant et al., 2017), sampling time in intertidal environments is limited and field work must be carried out within a short time frame (Drummond and Connell 2005; Parravicini et al., 2010). The *in situ* data collection method, which has been applied in large-scale studies in South America (e.g. NaGISA and SARCE, Cruz-Motta et al., 2020), requires personnel with expertise such as knowledge of local biodiversity, and that may be limited. The only way to increase sampling effort is to have more personnel and/or more field time both resulting in increased and usually unrealistic budgets. The photographic method proposed here requires low-tech, inexpensive and readily available equipment: basically, a quadrat and a simple digital camera with high resolution. Furthermore, field personnel require very basic training and photographic collection time is minimal. Later, photograph processing time will depend on the complexity of local species diversity and on the observer's expertise, with the advantage of not being field-time or weather restricted. Here, the method's taxonomic identification may be limited; however, our results suggest that these potential pitfalls do not seem to greatly affect community analysis results and overall findings.

Changes in biodiversity at different temporal and spatial scales are of central interest of large-scale monitoring programs (Hewitt et al., 2007). Techniques that allow for greater sampled area or replication within a site or a region are expected to generate the most reliable and

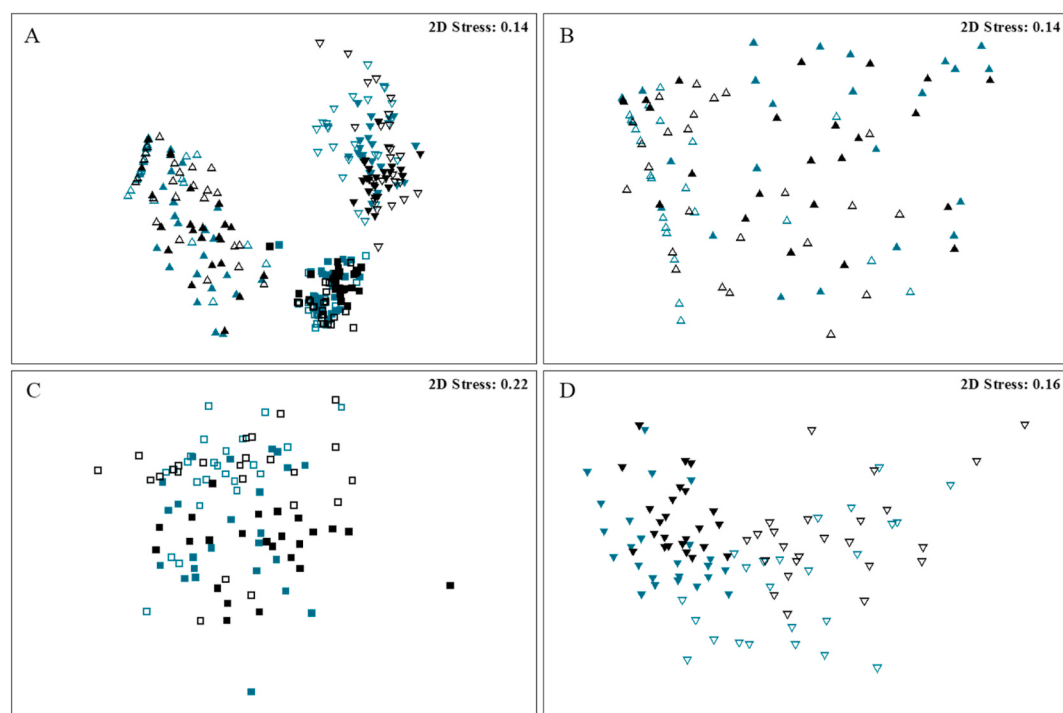


Fig. 1. non-metric MDS of cover data for 2 methods (visual quadrats: black, photoquadrats: grey) at 2 sites (Punta Este: filled shapes, Punta Cuevas: empty shapes) during 4 seasons (data pooled). A) 3 tidal levels (high intertidal, HT: ▲; mid intertidal, MT: ■; and low intertidal, LT: ▼); B) HT; C) MT and D) LT.

Table 1

2-Way PERMANOVA analysis of % cover of high, mid and low intertidal communities at the two sites. Me: Method and Se: Season. Values in bold are significant.

			Punta Este			
Source	df	SS	MS	Pseudo-F	P(perm)	perms
HT						
Me	1	1585.2	1585.2	0.88109	0.45	998
Se	3	36615	12205	6.7837	0.001	998
MexSe	3	2359.8	786.6	0.43721	0.936	998
Res	40	71966	1799.2			
Total	47	1.13E+05				
MT						
Me	1	340.15	340.15	2.008	0.092	997
Se	3	1003.9	334.65	1.9755	0.028	998
MexSe	3	299.83	99.942	0.58999	0.848	998
Res	40	6775.9	169.4			
Total	47	8419.8				
LT						
Me	1	1137.4	1137.4	3.6998	0.024	997
Se	3	9343.3	3114.4	10.131	0.001	998
MexSe	3	116.75	38.916	0.12659	0.998	999
Res	40	12296	307.41			
Total	47	22894				

			Punta Cuevas			
Source	df	SS	MS	Pseudo-F	P(perm)	perms
HT						
Me	1	2054.5	2054.5	1.5106	0.188	999
Se	3	23351	7783.6	5.7232	0.001	999
MexSe	3	3357.6	1119.2	0.82294	0.574	998
Res	40	54400	1360			
Total	47	83163				
MT						
Me	1	111.47	111.47	0.88208	0.393	999
Se	3	1297.8	432.59	3.4232	0.009	997
MexSe	3	670.6	223.53	1.7689	0.12	999
Res	40	5054.9	126.37			
Total	47	7134.7				
LT						
Me	1	2966.1	2966.1	4.2131	0.013	998
Se	3	22996	7665.2	10.888	0.001	999
MexSe	3	538.43	179.48	0.25493	0.995	999
Res	40	28161	704.01			
Total	47	54661				

Table 2a

Results of pair-wise comparison of methods within season of % cover data for LT of 2 sites.

Season				Site			
	Punta Este				Punta Cuevas		
	t	P(perm)	perms		t	P(perm)	perms
Summer	0.813	0.632	416		1.384	0.122	409
Autumn	1.243	0.151	416		1.237	0.184	407
Winter	0.905	0.478	405		1.034	0.325	412
Spring	1.178	0.256	401		0.999	0.412	401

Table 2b

Results of RELATE routine performed on % cover estimation for each method in the 3 tidal heights at the two sites. Similarity matrices among samples were used. Values in bold are significant.

Tidal height			Site		
	Punta Este			Punta Cuevas	
	ρ	<i>p</i> value		ρ	<i>p</i> value
HT	0.621	0.001	0.611	0.001	
MT	0.475	0.001	0.347	0.004	
LT	0.631	0.001	0.640	0.001	

representative results (Drummond and Connell 2005). The photographic method has often been used in subtidal reef surveys, recognizing possible biases (e.g., Rilov et al., 2018). We have shown that on the Atlantic Patagonian coast, this sampling method is as capable of detecting the spatial and temporal variability as the *in situ* method at various scales including tidal height, time of the year and site. Nonetheless, study designs on intertidal habitats that expand over broad latitudinal gradients should account for the differences in tidal amplitudes that generally exist from the tropics to the poles. High latitudes have large tidal amplitudes and well-defined vertical zonation, whilst low latitudes usually have small tidal amplitudes and tend to lack zonation. Thus, in order to standardize sampling methods in large-scale designs, a single intertidal level should be chosen and used across all latitudes. We suggest the use of the mid intertidal as the standardized sampling zone across latitudes on the basis of (1) this level would have more sampling time than the low intertidal within the sampling time restrictions imposed by the tides, (2) the mid intertidal is commonly more diverse than the high intertidal, and thus has a broader range of organisms to potentially respond to environmental changes, (3) the mid intertidal should be easily identified in locations with small tidal amplitudes and without zonation as an area between the high and low tide marks, and (4) the mid intertidal will be affected by climate change through changes in both the air (temperature, humidity and wind) and the ocean (warming and acidification).

Finally, scientifically and societally relevant data from large-scale and sustained monitoring are essential for conservation and management purposes. Stakeholders need to systematically assess the status of coastal biodiversity and ecosystems to improve their management policies. For example, through a better understanding of how coastal ecosystems are responding to increased human use and to climate change (Rilov et al. 2019, 2020b). Coastal monitoring activities will have the highest societal impact if they are (1) focused on measuring Essential Ocean Variables (EOVs) as identified by the Global Ocean Observing System (GOOS) (Miloslavich et al., 2018), because EOVs are general attributes of the system that can also be compared across systems and regions, and (2) contributing data into reporting structures such as the UN Sustainable Developmental Goals (SDGs, particularly SDG14), targets of the Convention for Biological Diversity (CBD) and the United Nations World Ocean Assessments (Miloslavich et al., 2016b). Key elements for sustainability include improving monitoring capacity through the implementation of agreed best practices, developing new automated measurements that facilitate data collection (for example, developing machine learning tools that can automate the analysis of the photo-quadrats), and enhancing capacity development and technology transfer (Bax et al., 2019). All of this will require a certain degree of investment which may be highly variable depending on each country's research and technical infrastructure, and socio-economic capabilities. Our study provides a first step towards improving capabilities in a developing region by providing empirical evidence that a simple, low-cost, non-destructive and low-tech method may provide the required information that large-scale monitoring programs need.

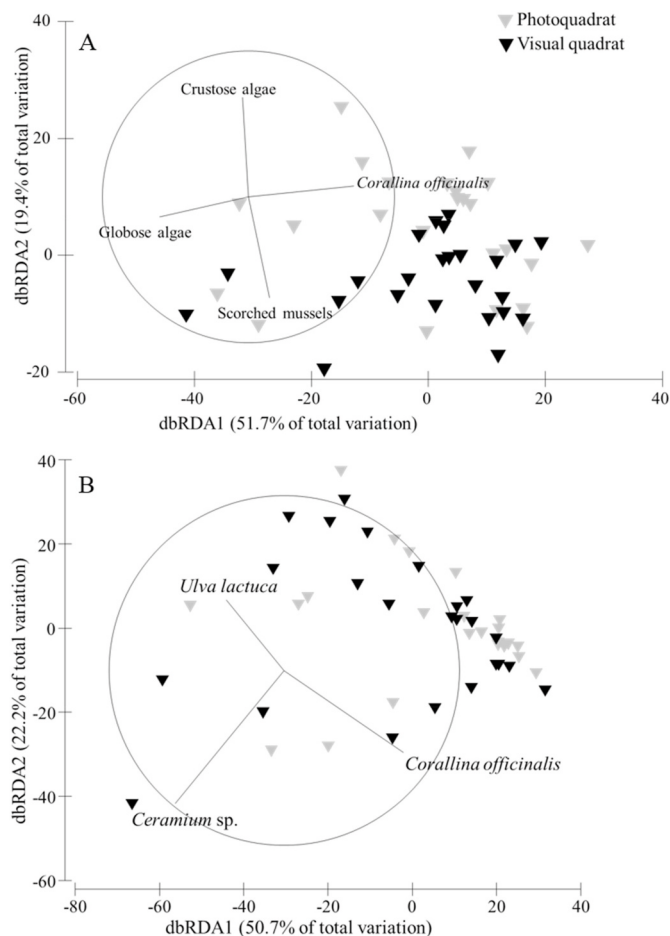
5. Conclusions

The results of this study demonstrated that the type of method used, photoquadrats vs visual counting in the field, slightly affected the measured rocky intertidal community structure only at the low shore level. Both methods were equally capable of detecting differences in communities among tidal heights and between sites. The species which best explained the observed variations were the same for both methods. Together, these results suggest that for the intertidal rocky shores of northern Patagonia both data collection methods lead to very similar results. The lower field-time needed to collect samples along with its low costs and simplicity, make the photographic method the most efficient. Thus, it is suggested for use in large-scale long-term monitoring of rocky shores.

Table 2c

Results of SIMPER analysis for each method in the 3 tidal heights (sites are pooled).

PQ						VQ				
	Species	Av.Abund	Av.Sim	Contrib%	Cum.%	Species	Av.Abund	Av.Sim	Contrib%	Cum.%
Average similarity: 55.30						Average similarity: 59.10				
HT	<i>Ulva prolifera</i>	1.95	43.56	78.76	78.76	<i>Ulva prolifera</i>	1.99	39.45	66.76	66.76
	Crustose algae	0.79	4.51	8.16	86.92	<i>Balanus glandula</i>	0.85	7.85	13.28	80.04
	<i>Balanus glandula</i>	0.66	4.40	7.95	94.87	Scorched mussels	0.72	6.91	11.69	91.73
Average similarity: 77.20						Average similarity: 73.28				
MT	Scorched mussels	3.00	45.47	58.90	58.90	Scorched mussels	2.97	38.05	51.93	51.93
	<i>Balanus glandula</i>	1.47	18.79	24.34	83.24	<i>Balanus glandula</i>	1.53	16.72	22.81	74.74
	Crustose algae	0.99	8.52	11.03	94.27	Crustose algae	1.03	7.71	10.52	85.26
Average similarity: 55.73						Average similarity: 58.22				
LT	<i>Corallina officinalis</i>	2.66	29.30	52.57	52.57	<i>Corallina officinalis</i>	2.59	27.13	46.60	46.60
	Crustose algae	1.13	7.89	14.16	66.73	Scorched mussels	1.52	12.77	21.93	68.53
	Scorched mussels	1.10	7.77	13.94	80.67	<i>Ulva lactuca</i>	0.84	4.68	8.04	76.56
	<i>Ulva lactuca</i>	0.68	2.79	5.00	85.67	Crustose algae	0.83	4.11	7.05	83.62
	<i>Undaria pinnatifida</i>	0.62	2.52	4.52	90.20	Globose algae	0.80	3.11	5.34	88.96
						<i>Ceramium</i> sp.	0.71	2.72	4.68	93.64

**Fig. 2.** DistLM analysis of LT communities sampled at A) Punta Este and B) Punta Cuevas. Vectors represent taxa that were correlated with plot axes.**Declaration of competing interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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