Application of a Simple, Low-Cost, Low-Tech Method to Monitor Intertidal Rocky Shore Assemblages on a Broad Geographic Scale

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Identifying susceptible regions where biodiversity changes occur at fast rates is essential in order to protect and ameliorate affected areas. Large-scale coastal monitoring programs that focus on long-term variability are scarce, yet the Marine Biodiversity Observation Network Pole to Pole is currently developing a regional collaboration throughout the American continent collecting biodiversity data in coastal habitats with a standardized systematic protocol. The use of photographic methods to collect assemblage data on intertidal rocky shores can be appropriate. The goal of this study was to analyze the performance of a simple, low-cost, non-destructive and low-tech photographic method on a broad geographical scale (∼ 2,000 km) of Atlantic Patagonian coastline. Concurrently, we aimed to identify indicators whose cover, presence or condition can be followed in time and used as beacons of change in biodiversity on these rocky intertidal shores. We also explored the potential relationships between assemblage structure and environmental variables, such as seascape classes. We identified and propose cover of mytilids, Corallina spp. and bare substrate as indicators of change due to their ecological relevance in intertidal assemblages and their visible and rapid response to human stressors or changes in environmental conditions. Finally, we illustrate the practicality and usefulness of remotely accessible environmental data, for instance the seascape classes approach as an integrative tool for large-scale rocky shore studies.

Keywords: biodiversity, rocky shores, monitoring, intertidal, Patagonia

INTRODUCTION

Coastal ecosystems generally present high biodiversity and provide valuable cultural, provisioning and regulating services (Galparsoro et al., 2014). Although they represent only 8% of global surface, these areas provide approximately 43% of the estimated value of ecosystem services worldwide. Concurrently, global average population density is estimated to be three times higher in coastal areas (Small and Nicholls, 2003) and these are also the most likely to be affected by natural hazards such as storm surges, hurricanes...
and other extreme weather events including those related to climate change (Kron, 2013). Within this context, rocky shores form more than 80% of coastal shorelines worldwide (Emery and Kuhn, 1982; Granja, 2004). Hence, monitoring for rapid changes to ecosystems on coastal rocky shores is imperative for adequate management and conservation of the services they provide.

Large-scale ecosystem monitoring programs on rocky shores are few despite the logistic advantages of these ecosystems (Miloslavich et al., 2019). Long-term, sustained, time-series of biodiversity, community structure and dynamics in this generally accessible ecosystem are few, such as MarClim in western Europe, PISCO on the west coast of the United States and SARCE in South America. Furthermore, data of the existing programs may be incomparable due to variability in the collection methods, heterogeneity in spatial and temporal sampling, and in data formats (Duffy et al., 2019; Miloslavich et al., 2019). Monitoring efforts at large spatial scales that try to integrate long-term inter-annual and seasonal community variability are scarce, mostly because they are costly, logistically complex and require much coordination by different groups of scientists. In this sense, contrasting access to resources of dedicated scientists involved in large-scale monitoring often challenges the implementation of programs at the desired scale (Bax et al., 2019). The implementation of large-scale and long-term monitoring programs is a tool for detecting changes in rocky shore communities that may provide early alarms to decision makers allowing the opportunity of a timely response action.

A recently established large-scale and long-term program is the Marine Biodiversity Observation Network Pole to Pole of the Americas (MBON P2P). It was conceived as an international network of collaborating research institutions, marine laboratories, parks, and reserves seeking to address common problems related to sustaining ecosystem services through conservation ecology. This project was built on the efforts of two previous international projects, namely the Natural Geography in Shore Areas (NaGISA) of the Census of Marine Life (CoML) program and its sequel for South America the South American Research Group on Coastal Ecosystems (SARCE) (Miloslavich et al., 2016). MBON P2P is collecting biological data in coastal habitats (rocky shores and sandy beaches) and acting as a global community of practice for sustained, operationalized measurements of marine biodiversity (Canonico et al., 2019). All data collected by the MBON P2P project are open available and contribute to other programs as the Global Ocean Observing System (GOOS- UNESCO), under the framework of Essential Ocean Variables (EOVs). Within this framework, some of the identified EOVs that are achieved by the MBON P2P program is “macroalgal canopy cover and composition,” as well as the emerging EOV “benthic invertebrate abundance and distribution,” both relevant to rocky shores (Miloslavich et al., 2018).

Along both Pacific and Atlantic South American coasts, the development of large-scale programs provided evidence of strong changes in community diversity (Cruz-Motta et al., 2020). Sea surface temperature (SST) was the main variable that explained the changes, yet local factors were also important (Cruz-Motta et al., 2020). Previously, similar programs also detected patterns in the distribution of the species assemblages on these coasts that were correlated with the SST and rainfall (Cruz-Motta et al., 2010). Atlantic Patagonia (41–55° S; 63–70° W) rocky intertidal shores were included as monitoring sites in the NaGISA-CoML and SARCE projects (Cruz-Motta et al., 2010, 2020; Rechimont et al., 2013; Miloslavich et al., 2016, among others) and are currently being included in MBON P2P. Communities within these habitats are exposed to particularly harsh environmental conditions that lead to adaptations of the local species (Bertness et al., 2006). In addition, a range of anthropogenic threats may add stress to communities and lead to changes in cover of dominant space occupiers (Mendez et al., 2017, 2021; Sorte et al., 2017).

Establishing a method that maximizes results and minimizes efforts is always a challenge for large-scale monitoring programs (Bax et al., 2019). Recently, a globally applicable and cost-effective method was tested for intertidal shore levels at two sites in southern Atlantic Patagonia by Livore et al. (2021). This method, non-destructive photographs of small parcels, showed similar results to others previously used for monitoring but required less time, knowledge and training in the field. The goal of the current study was to analyze that method’s performance on a broader geographical scale. To do so, this study sampled intertidal assemblages along ∼ 2,000 km of SW Atlantic Patagonian coastline using the afore mentioned method. The hypothesis was that the method would be capable of detecting differences among assemblages within two intertidal levels along the studied latitudinal scope as described by previous studies (Bertness et al., 2006; Rechimont et al., 2013; Raffo et al., 2014; Cruz-Motta et al., 2020). Furthermore, with the obtained data we identified biological indicators whose cover, presence or abundance can be followed in time through long-term monitoring programs on rocky intertidal shores. Finally, we explored potential relationships between the assemblages and relevant remotely sensed environmental variables (e.g., sea surface temperature, chlorophyll-a and seascape classes) as an illustration of their usefulness in long-term monitoring.

**MATERIALS AND METHODS**

**Study Area**

Sampling was carried out on the rocky intertidal shores of Atlantic Patagonia from Estancia San Lorenzo, Chubut (42.094° S; 63.910° W) to Ushuaia, Tierra del Fuego (54.849° S; 68.494° W). Nine locations were sampled along >2,000 km of coast (Figure 1). Rocky intertidal platforms sampled were exposed to semidiurnal tides with tidal amplitudes that ranged from 4 to 9 m. All locations presented an intertidal biological zonation: high (HT), mid (MT) and low (LT) intertidal. At some locations the HT were covered by pebbles and were impossible to sample, hence only MT and LT were included in this study. The MT was generally dominated by a matrix of mytilids that may include the scorched mussels *Brachidontes rodriguezii* and *Perumytilus purpuratus* as well as *Mytilus edulis*. The LT was generally characterized by several algal species including a large proportion of calcareous algae *Corallina* spp. as well as gastropods *Tegula*...
**Sampling**

Samples were collected between February and May 2017 during diurnal low tides. Percentage cover of sessile organisms was estimated from high definition photographs of $25 \times 25 \text{ cm}^2$ quadrats haphazardly placed on the substrate ($n = 15$ per level and location; $n = 255$, the LT of location San Julian could not be sampled). The compact camera (Nikon Coolpix AW 130) was fixed on a purposely built pvc-tube stand to standardize distance from the substrate. No zoom was used and the same setting was used throughout sampling. Photographs were analyzed using the free software Coral Point Count (CPCe V 4.1, Kohler and Gill, 2006). One hundred equidistant points were placed over the digital image and sessile organisms observed under each point.
were registered to estimate cover. All organisms were identified to the lowest possible taxonomic level. Concurrently, all mobile organisms larger than ∼1 cm observed within the quadrat were also determined to the lowest possible taxonomic level and counted. For analysis, *Perumytilus purpuratus*, *Brachidontes rodriguezii*, *Aulacomya atra*, and *Mytilus edulis* were grouped within the category Mytilids, as identification to species level through photographs is unreliable. The same occurred for the red algae *Ceramium* sp. and *Polysiphonia* sp. and the pulmonate limpets *Siphonaria lessonii* and *S. lateralis*.

**Environmental Variables**

Following the results of the SARCE project (Cruz-Motta et al., 2020), sea surface temperature (SST), chlorophyll-α concentration, photosynthetic active radiation (PAR), daily rainfall and air temperature, were extracted from different remote sensing sources (see Supplementary Table S1 in the Supplementary Material). Data from 1 January 2016 to 31 May 2017 was used for all variables. Virtual stations were located up to 5 km offshore of the sampling station to avoid land interference and the land mask imposed by some of the products. The spatial resolution varied according to the data source, from 1 degree (air temperature) to 1 km [Multi-sensor Ultra-high Resolution (MUR)]. We included the novel seascape source, from 1 degree to 1 km [Multi-sensor Ultra-high Resolution (MUR)]. We included the novel seascape classification of the marine environment, as a multiscale, and synoptic characterization of the SST, salinity, chlorophyll-α and chromophoric dissolved organic matter (CDOM) represented as a catalog of classes (Kavanaugh et al., 2016).

**Data Analysis**

Non-metric multi-dimensional scaling (nMDS) was used to visualize multivariate patterns in benthic assemblages cover at various scales. Benthic assemblage cover data were analyzed separately for each location 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 on square-root transformed data for the analyses, which used 9,999 permutations of residuals under a reduced model (Anderson et al., 2008). PERMANOVA model had two factors: Location (Lo, random, 9 levels) and Intertidal Level (IL, fixed, 2 levels: MT and LT). Pairwise comparisons were performed among all pair of locations within each level to identify differences. SIMPER analyses were used to determine which taxa contributed more to similarity within samples. DistLM analyses were performed MT and LT assemblage data to identify and visualize taxa that contributed most (>0.60 correlation) to the observed differences for each level.

A Kruskal-Wallis test was used to analyze differences in cover of the dominant categories for each intertidal level (i.e., Mytilids and bare substrate for MT and Corallina algae and bare substrate for LT), as they may be used as Environmental Ocean Variables in monitoring programs.

Abundances of mobile organisms that were recorded at five or more locations and exceeded a mean of 10 individuals m⁻² across all locations, were compared through Kruskal-Wallis to analyze differences among locations. Taxa that were recorded at fewer than five locations or had mean abundances <10 individuals m⁻² were not considered.

A correlation analysis between the Bray Curtis similarity matrix and the environmental variable matrix (using Gower similarity index) was performed with the BioEnv algorithm to establish potential relationships between both ordination arrangements (Clarke and Ainsworth, 1993).

**RESULTS**

The photoquadrat protocol used in this study detected a total of 26 taxa for which cover was estimated from the MT and LT levels across more than 2,000 km of Atlantic Patagonian intertidal rocky shores. Four of these taxa were sessile invertebrates that include four species of mytilids (*Brachidontes rodriguezii*, *Perumytilus purpuratus*, *Mytilus edulis*, and *Aulacomya atra*) which were grouped for analysis and three species of barnacles (*Notobalanus flosculus*, *Notochthamalus scabrosus*, and *Balanus glandula*), whilst the remaining taxa were macroalgae.

The assemblages from the MT level were significantly different from the assemblages from the LT and among sampling Locations [PERMANOVA. LoxIL: pseudo-F = 27.82, df = 7, p(perm) < 0.001]. Pairwise comparisons showed that intertidal levels within each location differed significantly [all comparison (p(perm) > 0.001)]. In the same way, nm-MDS analysis showed separation of samples by intertidal level, with the LT of Ushuaia as the only assemblage that was clearly separate from all others (Figure 2). A high similarity within the studied range of both MT and LT was detected, with 84.92 and 76.71%, respectively. Within each level very few categories explained >80% of the dissimilarity (Table 1). Those categories were Mytilids and to a lesser degree bare substrate in the MT. In the LT *Corallina* spp. was consistently the most explanatory category with several
TABLE 1 | Results of SIMPER analysis for each location in the two tidal levels.

| Location | MT                  | LT                  |
|----------|---------------------|---------------------|
| ESL      | Mytilidae Av. similarity: 95.79 | Coralina spp. Av. similarity: 73.19 |
|          | 9.89 Av. Abund 82.31 Av. Sim 85.93 Contrib% 85.93 Cum.% | Ulva spp. 5.37 26.17 35.75 95.61 |
| PLE      | Mytilidae Av. similarity: 92.27 | Coralina spp. Av. similarity: 83.75 |
|          | 9.68 70.89 76.83 76.83 | Bare substrate 4.32 21.26 25.39 73.98 |
|          | Bare substrate 1.89 10.66 11.56 88.38 | Ulva spp. 2.45 10.04 11.99 85.98 |
| PLE      | Mytilidae Av. similarity: 79.08 | Coralina spp. Av. similarity: 86.88 |
|          | 9.55 65.59 82.94 82.94 | Ulva spp. 2.06 10.15 11.20 84.15 |
| CAM      | Mytilidae Av. similarity: 85.53 | Ulva spp. 2.96 10.04 11.99 85.98 |
|          | 9.41 71.39 84.1 84.1 | Bare substrate 4.32 21.26 25.39 73.98 |
| PBU      | Mytilidae Av. similarity: 81.93 | Bare substrate 4.32 21.26 25.39 73.98 |
|          | 9.79 75 91.54 91.54 | Ulva spp. 2.96 11.71 15.36 68.87 |
| SJU      | Mytilidae Av. similarity: 87.71 | Bare substrate 2.96 11.71 15.36 68.87 |
|          | 9.41 71.84 81.9 81.9 | Ulva spp. 2.45 10.04 11.99 85.98 |
| MLE      | Mytilidae Av. similarity: 86.79 | Ulva spp. 2.45 10.04 11.99 85.98 |
|          | 9.05 65.34 75.29 75.29 | Bare substrate 4.32 21.26 25.39 73.98 |
|          | Bare substrate 3.78 21.12 24.34 99.63 | Mytilidae 3.38 15.37 18.71 80.1 |
| RGR      | Mytilidae Av. similarity: 79.88 | Mytilidae 3.6 10.23 15.6 71.57 |
|          | 9.2 63.68 79.72 79.72 | Ulva spp. 2.96 11.71 15.36 68.87 |
|          | Bare substrate 2.8 11.8 14.77 94.49 | Ceramium sp. 2.73 9.75 12.78 81.65 |
| USH      | Mytilidae Av. similarity: 75.27 | Mytilidae 3.6 10.23 15.6 71.57 |
|          | 9.2 59.16 78.59 78.59 | Ulva spp. 2.45 10.04 11.99 85.98 |
|          | Undetermined algae 1.15 5.34 7.09 85.88 | Ceramium sp. 2.73 9.75 12.78 81.65 |
|          | Bare substrate 5.04 19.6 27.21 27.21 | Notochthamalus scabrosus 4.2 14.81 20.56 95.69 |

Cover in MT was consistently dominated by mytilids with average cover ranging from 82 to 98%, yet differences were detected among groups ($H = 32.917, df = 8, p < 0.001$) (Figure 3). Bare substrate cover differed within the studied geographical range ($H = 52.978, df = 8, p < 0.001$) and ranged from 0.27 to 16.53% (MLE > PMA = SJU = RGR > USH > PLE > CAM > PBU > ESL). In the MT bare substrate (50%), the alga Pyropia sp. (14%) and the two barnacles Balanus glandula (11%) and Notochthamalus scabrosus (10%) explained 85% of the observed variation (Figure 4A).

Cover in the LT was generally dominated by the alga Corallina spp. except in USH where mytilids had larger cover (mean: 21.33%). Corallina spp. cover, when present, ranged from 60 to 91% and differences among groups were detected ($H = 69.530, df = 7, p < 0.001$) (Figure 3). Bare substrate cover also differed within the studied area ($H = 85.123, df = 7, p < 0.001$) and ranged from 27.80 to 0.40% (USH = PLE > MLE > PMA = PBU > RGR > CAM = ESL). In the LT Corallina spp. (47%), Ulva spp. (18%), Ceramium sp. and
Polypsisiphonia sp. (10%), mytilids (8%), and bare substrate (7%) accounted for 90% of the observed variation (Figure 4B).

A total of 13 taxa of mobile macroinvertebrates were found within all the study locations: the sea anemones Parabunodactis imperfecta and Bunodactis octoradiata, the gastropods Siphonaria lessonii, S. lateralis, Nacella magellaniaca, Pareuthria fuscata, Tegula patagonica, Fissurella radiosa, and Trophon geversianus, undetermined chitons that were grouped, the isopod Exosphaeroma sp. and the sea urchin Arbacia dufresnii. Abundances of only Siphonaria spp. (S. lessonii and S. lateralis) in MT and N. magellanica in LT were of relevance (mean abundance > 10 individuals m$^{-2}$) for statistical analysis. Abundance of Siphonaria spp. ranged from 0 to 512 individuals m$^{-2}$ and differed among locations ($H = 113.282, df = 8, p < 0.001$. SJU > MLE = USH > PLE > CAM = PMA = PBU > ESL = RGR). N. magellanica ranged from 0 to 204 individuals m$^{-2}$ being highest in USH and different than all other locations ($H = 77.253, df = 7, p < 0.001$).

Three seascape classes were observed in the region during the studied period (Table 2). Among all the studied environmental variables, the number of seascape classes alone, number of seascape classes and PAR, and SST, number of seascape classes and number of switches between seascape classes were the models that best explained assemblage variability present at each sampling location during the 2017 summer ($\text{BioEnv } r = 0.60, 0.59, \text{ and } 0.56$, respectively).

**DISCUSSION**

The results of this study demonstrate that the simple, low-cost, low-tech method used here is capable of detecting differences in assemblage structure along an extension of ~2,000 kilometers of Atlantic Patagonian coast. Differences among assemblages from two levels of air exposure within this broad geographic scale were clearly identified. Despite the low variability in each exposure level, the method was able to detect differences among assemblages and in the dominant taxa percentage cover. The method allows for the adequate collection of useful cover data of sessile species and abundance of some slow moving taxa which can be linked to remotely accessible environmental data.

Visual methods performed in situ, such as the NaGISA protocols (Rigby et al., 2007) used in previous large-scale programs, can take up to 2 days at a single site to obtain the desired data whilst the method employed here required a single day. This non-destructive method can be repeated at the same locations throughout an extended period of time and can be applied by scientist globally due its simplicity and low cost. Concurrently, it is suitable to determine changes in cover or abundance in several indicators as reported here. Identification of organisms to a low taxonomic level and the impossibility of viewing primary cover when dense algal stands are present are the most important limitations the used method has (Livore et al., 2021). Hence, for detailed description of intertidal communities, such as species...
richness and diversity, this may not be suitable. However, for large-scale long-term studies focusing on major changes in primary space occupying species this method should be broadly applicable.

This study identified taxa whose changes in cover largely explained the changes in assemblage structure at the two described intertidal levels. For the mid intertidal mytilids, bare substrate and *Balanus glandula* were the variables that better explained the observed patterns, whilst for the low intertidal *Corallina* spp., bare substrate and mytilids, explained most of the variability. We propose mytilids and *Corallina* spp., in particular, as biological indicators for broad scale monitoring.
programs because they are cosmopolitan taxa with a conspicuous presence, they have a low probability of misidentification, they are ecologically relevant (i.e., habitat forming species that sustain many other dependent species) and they respond to human stressors or changes in environmental conditions (Kelaher, 2003; Liuzzi and López Gappa, 2008; Buschbaum et al., 2009; Olabarria et al., 2016; Vinagre et al., 2016). Concurrently, bare substrate is proposed as another indicator because changes in this variable reflect either settlement or disappearance of primary space holders, which are often also habitat forming species. Hence, changes in the relative cover of bare substrate directly indicate changes in assemblage structure (Pickett and White, 2013; Mendez et al., 2019). Two algae, Pyropia sp. and Ulva spp. also contributed to the observed patterns in the mid and low intertidal, respectively. However, the high natural fluctuation along with the limited distribution of these taxa suggest they may be less appropriate indicators for long-term, large-scale studies (Raffo et al., 2014). There may be other suitable indicators that may be considered upon application of the method along the American coastline.

The use of this simple method also allows the possibility of relating assemblages with remotely obtained environmental variables. This study was able to detect a correlation between seascape classes and biotic assemblages of individual locations. Linking environmental variables collected from remote sensing platforms offshore the sampling sites may represent a potential source of error (Turner et al., 2003). However, this feature provides easily accessible environmental data for remote locations where almost no local continuous environmental data exists. The global seascape classes appear as a very promising environmental predictor as they summarize the interaction and variability in time of multiple variables in a typology of pelagic realms (Kavanaugh et al., 2016). The correlation between the classes and the community structure of pelagic communities has recently been demonstrated (Montes et al., 2020). However, this is the first time that pelagic seasapes are related to rocky shores assemblages. The alternation between the predominant classes might result in a robust predictor for the changes in cover/abundance of the main groups that form the intertidal coastal communities. In this sense, long-term monitoring programs will provide replication in time of assemblage data and seascape information which combined, as in this study, can be used as a synoptic and integrating assessment tool.

The macroecology approach brought together ecology and biogeography in the early 1990s in the sense that ecologists recognized that external influences may strongly affect community structure, whilst biogeographers acknowledged that community events may have broad significance on distribution patterns (Briggs, 2007). Within this framework, regional scale studies with consistent and extensive local sampling that describe assemblages are needed to appropriately address ecological process and geographic distribution. Without this knowledge there is a risk of a continuous description of heterogeneity without the capability of integration to address broader scale problems (Connell and Irving, 2008). The current study is a step in that direction, describing dissimilarities in assemblages along ~ 2,000 kilometers of largely unexplored coasts of Atlantic Patagonia. In our study changes in the assemblages were moderately associated to four environmental variables. The lack of a more distinct pattern in the studied assemblages could be suggesting that biodiversity of these rocky intertidal habitats is likely driven by a combination of factors that operate at different spatiotemporal scales as has been suggested for other marine communities (Witman et al., 2004; Connell and Irving, 2008; Cruz-Motta et al., 2020).

Whilst recent efforts are encouraging and supporting long-term and large-scale monitoring programs that integrate scientist at the continental level, they are still scarce (Canonico et al., 2019 and references therein). This could be due, at least in part, to the difficulties of finding methodologies that are both scientifically robust, practically achievable and logistically inexpensive in order for it to be accessible and feasible to a wider range of potential participants (Stephenson et al., 2017). The method employed here complies with all the above mentioned attributes and is therefore suggested for use in long-term and broad-scale monitoring programs such as MBON P2P. Its application within the MBON P2P will provide information on the associations among assemblages along the American coastline and test the proposed ecological indicators. When coupled with environmental variables that are remotely and easily accessible they could provide the tools for an integrative approach to an informed coastal management.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**AUTHOR CONTRIBUTIONS**

JL conceived the project, participated in data collection in the field and lab, data processing, and wrote the manuscript with contribution from all authors. MM conceived the project,
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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2021.589489/full#supplementary-material

Supplementary Table 1 | List of environmental variables used in analyses.
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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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