VIE ET MILIEU - LIFE AND ENVIRONMENT, 2020, 70 (2): 141-147

TIME-CONSTRAINED SAMPLING: A LITTLE-EXPLORED ALTERNATIVE FOR MARINE HARD BOTTOM COMMUNITIES

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HARD BOTTOMS
MARINE BENTHIC COMMUNITIES
METHODS FOR SAMPLING
BENTHIC ORGANISM SAMPLING

ABSTRACT. – Marine hard bottoms support the highest proportion of marine biodiversity. In addition, there are a wide range of sizes and forms among the benthic species of a hard bottom community: solitary or modular, from small organisms to large erected colonies, epibionts, encrusting fauna, mobile predators, etc. These habitats and their biological communities have a high heterogeneity at different spatio-temporal scales. The high diversity and heterogeneity of the hard bottom habitats can present considerable difficulties to obtain accurate and statistically comparable data for community monitoring. Thus, marine hard bottoms are complex habitats where monitoring protocols vary considerably. We review the main approaches used in the study of this type of habitat, assessing some of their main advantages and disadvantages, and highlight a not sufficiently explored alternative where the sampling effort is controlled by a set period of sampling time. Time-constrained searches have been routinely used in terrestrial ecological studies but few explore in marine habitats. However, it is an acceptable alternative that would be worth further exploring for studding marine hard bottom communities. Time-constrained methodology presents a good cost-benefit balance: It provides a good representation of the diversity of hard bottom communities, the costs (both in time and economic resources) for sampling and sorting are lower, and the quantification of taxa allows their relative abundance to influence the results.

INTRODUCTION

Rocky coastal habitats represent a quantitatively small portion of the marine environment in comparison to the spatial extent of the soft bottoms; however, they support the highest proportion of marine biodiversity (Steneck *et al*. 2002, Wieters *et al*. 2003, Bianchi *et al*. 2004, Wahl 2009). Marine hard bottoms are complex habitats where monitoring protocols vary considerably (Van Rein *et al*. 2009, Beisiegel *et al*. 2017). The choice of methodology has strong implications for the efficiency of the sampling, the monitoring efforts and analysis; the level of precision, the time spent in the field, etc. (Hewitt *et al*. 2001, Smale *et al*. 2012, Beisiegel *et al*. 2017). The final selection of methodology is done as necessary involving various practical considerations, such as: the time and the budget available to sort and identify the samples; the inherent complexity of the habitats studied (depth, hydrodynamism, topological heterogeneity, environmental conditions in general, etc.); the difficulty in handling the quadrat or any other tool used to delimit the sampling unit; among many others. In any case, being conscious of the strengths and the weaknesses of a selected methodology is important for interpreting the results of studies within reasonable limits. Here we review, together with the problems of sampling in marine hard bottoms, the main approaches used in the study of this type of habitat assessing some of their main advantages and disadvantages (Table I), and highlighting a few explored alternatives where the sampling effort is controlled through a set period of sampling time.

Unlike sedimentary habitats, marine hard bottoms are highly heterogeneous (cliff, caves, crevices, reefs, big rocks, etc.) (Bianchi *et al*. 2004, Bulleri & Chapman 2004, Fraschetti *et al*. 2005), and they are inhabited by highly heterogeneous communities at many spatial scales, from hundreds of kilometers to centimeters (Archambault & Bourget 1996, Blanchard & Bourget 1999, Fraschetti *et al*. 2001, Anderson *et al*. 2005a). There is a wide range of sizes and forms (and consequently, densities) among the benthic species of a hard bottom community: solitary or modular, from small organisms (few millimeters) to large erected colonies, epibionts, encrusting, mobile fauna, etc. (Gray 1997, Bianchi *et al*. 2004, Wahl 2009). This fact can present considerable difficulties to obtain accurate and statistically comparable data for community monitoring (Munro 2005, Van Rein *et al*. 2009). The high diversity and heterogeneity of the hard bottom habitats imply difficulty for studying biological communities since every sample should represent the studied community (Hurlbert 1984, Underwood 1997, Quinn & Keough 2002, Benedetti-Cecchi 2003, Murray *et al*. 2006a). For these reasons, it is very difficult to study all components of hard bottom benthic communities; the available methodologies rarely allow the gathering of representative samples

Table I. – Main characteristics of different methodologies in marine hard bottoms.

Method	Advantages	Disadvantages
Standardized small sampling unit. Scraping the whole surface in the sampling unit	Precise quantification of the organisms in sampling unit	Does not represent the whole species and microhabitat diversity
	It facilitates the between site and between studies comparisons, regardless of different collectors	High sorting time
Image capture. Video and Photo survey	Rapid rate of data collection and low cost	Not particularly suitable for mobile fauna or erected organisms
	Permanent record of the samples	Many organisms cannot be identified only by image or visually
	No impact on ecosystem	
	Repeatability	
	Underwater work fast	
Combination of quadrats and semi-quantitative estimation of abundance	Can deal with large sampling units	Not a precise quantification of all organisms inside each sampling unit
	It considers both modular and individual organisms	Usually, only suitable for visually identifiable organisms
Rapid Assessment Survey	It is a non-destructive methodology because it is mainly qualitative	Not a precise quantification of the organisms
	It is not restricted to a specific sampling unit	Risks of subjectivity in taxonomy (It is necessary a previous training)
	Large sampling areas can be surveyed	Usually, the primary aim is to detect only alien species
	Low cost	
Time-constrained sampling	Good representation of species diversity, detection of inconspicuous and rare species	Not a precise quantification of all organisms inside each sampling unit
	Large sampling unit can be sampled and processed in laboratory	It is necessary a previous training
	The quantification of taxa in laboratory allow their abundance to influence the results	

for all components and, in practice, the researchers often select only a part of the whole community. This selection can be made based on different criteria: taxonomy, sizes, trophic groups, life forms, to study only visually detectable organisms, etc. Nevertheless, even if the researchers resolve to study only a part of the whole community, there is another crucial decision: the type and size of the sampling unit.

Plots of standardized areas

A common option is to use a standardized sampling unit (quadrat, rectangles, circles, etc.) with a dimension that allows a precise quantification of all organisms within this unit, typically 1 × 1 m, 50 × 50 cm, 25 × 25 cm, etc. It is usually assumed that this method allows a precise estimation of abundance and density in the community. Using this method, samples usually do not represent fully the whole species and microhabitat diversity of a given area (except, perhaps in the simplest communities) (Chapman & Bulleri 2003, Bulleri *et al*. 2005, Pister 2009), due to under-sampling of uncommon and rare species (Dethier *et al*. 1993, Miller & Ambrose 2000, Murray *et al*. 2006a). Several authors recorded this fact for benthic hydroid assemblages (Boero & Fresi 1986, Piraino *et al*. 2013). This approach is a standard methodology widely used in marine ecology (Cochran 1977, Munro 2005, Murray *et al*. 2006b, Thompson 2012), but it ignores the problems that derive from unrepresentative samples, unless for a part of the community. For example, unrepresentative samples can often result in high variability (high dispersion) between samples. In these cases, it could be more difficult to find patterns and ultimately, the null hypothesis may be retained when it could be false (Type error II) (Underwood & Chapman 2003, Murray *et al*. 2006a).

One additional source of imprecision in this methodology is the fact that plots and quadrats only have two dimensions and cover an area of substratum that frequently is highly heterogeneous in three dimensions. Thus, the real sampling area under the projection of a sampling quadrat is unknown, variable and greater than the quadrat area itself (Murray *et al*. 2006b). Furthermore, in very heterogeneous habitats such as marine hard bottoms,

the selection of the precise position of the frame can be crucial. This fact could lead the researcher to choose the position of the sampling units by avoiding crevices, big rocks or some other microhabitats that could make the sampling protocol more complicated. This is, in practice, a selection of a part of the community that is seldom documented in the methods. If the objective is to sample the complete community, the randomization of sampling unit placement ensures that estimations are unbiased and that statistical inferences are reliable (Quinn & Keough 2002). However, the use of these sampling units can often be complicated, for instance, if a unit falls in a deep crevice. A non-random (or at least haphazard) sample makes it difficult to extrapolate accurate results or to even obtain general conclusions.

Increasing the sampled area

Researchers can use different strategies to obtain a more comprehensive representation of microhabitats and rare species, such as increasing the number and/or size of the sampling units (*i.e.*, increasing the sampling effort) (Chapman 2003, Murray *et al*. 2006b). The first option, enlarging the number of samples, does not involve enlarging the representation of taxa in each sampling unit. The variability among sampling units within the cells of the experimental design can be large, which can pose some difficulties in subsequent analyses. But, unless, the probability of finding and representing different types of microhabitats and associated organisms increases with a greater number of samples.

In any case, bigger and/or more numerous samples cannot be sorted in a quantitative manner without a much larger (and probably unpractical) effort (Chapman 2003, Piraino *et al*. 2013), and they are sometimes processed on a presence/absence basis without comparison of relative abundances (Chapman 2003, Chapman & Bulleri 2003, People 2006, Glasby *et al*. 2007, Pister 2009). Only for large and visually classifiable organisms do the use of large sampling areas properly represent the spatial heterogeneity and are compatible with a precise quantification (for instance, Nadon & Stirling 2005, Villegas *et al*. 2008, Cúrdia *et al*. 2013). Another important point is that, in subtidal studies, an increment of the sampling effort is limited by scuba diving time, particularly at depths greater than 15-20 m. Furthermore, a complete collection for such a large surface area would make the sampling very destructive, which should be generally avoided and would be unacceptable in Marine Protected Areas. For this reason, where the organisms under study are visually classifiable (to the categorical level chosen), a non-extractive sampling could be used.

Sampling using image capture has grown in the last years (video, photo, etc.) (Kohler & Gill 2006, Van Rein *et al*. 2011, 2012, Howarth *et al*. 2015). The benefits of this methodology are mainly its rapid rate of data collection and low cost, as well as the ability to create a permanent record of the samples, and the possibility of collecting repeated samples through time (Underwood & Jackson 2009). However, many erect organisms (such as algae canopies, large colonies of hydroids, gorgonians, corals, etc.) have a three dimensional development with projections, secondary stems, etc., and they can overlie the organisms attached to the primary substratum (Bianchi *et al*. 2004, Underwood & Jackson 2009), which could easily be underestimated. In addition, these methods are not particularly suitable for mobile fauna. Furthermore, many organisms cannot be identified only by image or visually, either because of the level of classification chosen, it is necessary that their observation is carried out under stereoscope and microscope (*e.g.*, spicules of sponges, nematocysts of hydroids, zooidal aperture in cheilostomate bryozoans, etc.), and/or because they are too small (such as a few millimeters in size). In these cases, as well as for the study of infaunal species inhabiting rocks, biogenic structures or other organisms (such as kelp holdfasts), an extractive sampling method is required (Thiel & Vásquez 2000, Anderson *et al*. 2005b, Sato-Okoshi *et al*. 2008, Tuya *et al*. 2011).

NEW APPROACHES TO DEAL WITH LARGE SAMPLING UNITS

As stated before, to obtain samples that reasonably represent microhabitats and species diversity in highly heterogeneous hard bottom environments, it is necessary to explore a rather large area. However, it can be very difficult (and often impractical) to obtain, sort and quantify these samples using traditional extractive methods (scraping the whole surface or collecting every single specimen in the explored area).

To address this problem, some authors have developed methodologies for faster quantification using a combination of quadrats and a semi-quantitative estimation of abundance (see Fraschetti *et al*. 2001, Bianchi *et al*. 2004, Parravicini *et al*. 2010, Airoldi *et al*. 2015). These methodologies consider both modular and individual organisms. Large sized organisms can easily be counted in more than one subquadrat, as well as smaller but more abundant ones. Other semi-quantitative methods are the "SACFOR-type" methodologies (Connor *et al*. 2004, Fariñas-Franco & Roberts 2013). These methodologies provide an ordinal estimation (ordered categories) of the relative abundance of the organisms. Although these semi-quantitative methodologies are often only suitable for visually identifiable organisms, they are valid and useful in the study of hard bottom benthic communities.

In addition to these semi-quantitative methodologies, some authors propose the use of visual techniques that reduce the sampling time but ensure a sufficient representation of the diversity of organisms (Boero & Fresi 1986,

Piraino *et al*. 2013). This methodology often implies prior selection of certain components of the community; these authors developed this methodology specifically for benthic hydroids, and it was further used by several authors in temperate and tropical zones (Di Camillo *et al*. 2008, Puce *et al*. 2009, González-Duarte *et al*. 2013, 2016, Megina *et al*. 2013). Several authors have pointed out that visual samplings are robust with regard to the error induced by operators (Dethier *et al*. 1993, Benedetti-Cecchi *et al*. 1996, Piraino *et al*. 2013). This methodology provides a good representation of species diversity, sampling inconspicuous and rare species (Boero & Fresi 1986, Murray *et al*. 2006b, González-Duarte *et al*. 2013, 2016, Megina *et al*. 2013, Piraino *et al*. 2013). These rare species could be essential in the characterization of communities and ecosystems (Boero 1994, Piraino *et al*. 2013). Moreover, this methodology provides an estimation of the abundance of the organisms under study, which would otherwise be impossible to process because of the large size of the sampling unit.

A way to make this methodology more standardizable is to constraint the sampling effort by previously established times, a methodology that has not been much explored in marine benthic studies, specially in subtidal habitats. Time-constrained searches have been routinely used in terrestrial ecological studies (Corn & Bury 1990, Will-Wolf *et al*. 2002, Berryman & McCune 2006, Marsh 2009). The sampling is carried out in a large sampling area during a precise previously defined time period. The time should be long enough to ensure a good qualitative and quantitative representation of the community studied but not so long that all organisms present are sampled; this methodology is practically applicable. By means of a preliminary study, an optimal sampling time can be defined according to the objectives (assessment of the biodiversity, trophic groups, assemblages compositions, etc.). Indeed, some authors have already used an approximation of this methodology. For example, in the study of the sessile communities associated with marinas, several authors carried out a time-constrained sampling for benthic ascidians (Airoldi *et al*. 2015) and benthic hydroid assemblages (Megina *et al*. 2016). Cohen *et al*. (2005) used this methodology for sampling the complete benthic hard bottom community in commercial ports.

Actually, in the context of bioinvasion studies, some authors have also developed methodologies for a faster sampling of benthic community associated to floating pontoons, marinas and artificial habitats in estuarine zones (Arenas *et al*. 2006, Ashton *et al*. 2006, Campbell *et al*. 2007, Bishop *et al*. 2015, Nall *et al*. 2015). In this methodology, names as Rapid Assessment Survey, previously trained sampling teams survey the benthic community in the study zone during a pre-defined sampling time (Arenas *et al*. 2006, Bishop *et al*. 2015). This method can be strictly visual sampling (Campbell *et al*. 2007), some specimens can be collected for laboratory identification of species (Arenas *et al*. 2006) or including a semi-quantitative estimation of abundance of each species encountered (Bishop *et al*. 2015). Furthermore, the sampling team may define some target species (*e.g*., alien species) (Minchin *et al*. 2006, Minchin 2012, Nall *et al*. 2015) and to focus on, instead of the whole community.

González-Duarte *et al*. (2013), in the study of benthic hydroid assemblages in well preserved natural zones, also carried out an approximation of this methodology. Samples are collected by SCUBA divers along transects of 25 m in length. Each transect is subdivided in five large rectangular sampling unit with a width visually controllable by a diver (1 m) and a length of 5 m, where previously trained divers swim at constant speed. The sampling time on each sampling unit is defined previously. The operators collect while swimming all substrates or potential substrates for hydroids (for instance algae, barnacles, sponges, etc.), as well as detectable colonies and patches of colonies they could detect in the time previously defined. Later, the total number of colonies collected is sorted and counted for each sampling unit. This methodology provides a good representation of species diversity, sampling inconspicuous and rare species (Boero & Fresi 1986, González-Duarte *et al*. 2013, 2016, Megina *et al*. 2013, Piraino *et al*. 2013). González-Duarte *et al*. (2013, 2016) sampled the 52 % of all Leptothecata species, with a know benthic phase, described for the Mediterranean Sea and the Strait of Gibraltar, and the 21 % of all Anthoathecata species (see Bouillon *et al*. 2004). Thus, this methodological line could be an acceptable alternative that would be worth to explore further and it can thus be chosen for study marine hard bottom communities on large areas.

A priori, these new approaches for the study of marine hard bottoms may seem less accurate and less precise in the quantification of the components of the samples than those more standardized and common methodologies (*e.g*., small and manageable sampling units). Nevertheless, most studies about benthic communities analyze the obtained data of abundances after a square root, fourth root or log transformation (Connell & Glasby 1999, Glasby & Connell 2001, Fraschetti *et al*. 2002, Anderson *et al*. 2004, Walker & Schlacher 2014, Uribe *et al*. 2015). This converts the precise quantitative information in a kind of semi-quantitative data scale. For example, a range of abundances between 0 and 700 individuals will be reduced by approximately 0-26.5 in a square root transformation; 0-5.1 in a fourth root transformation and 0-6.6 in a log transformation. Differences between 600 and 700 individuals in two species would be reduced by a difference of 24.5 to 26.5 in a square root transformation, by 4.9 to 5.1 in a fourth root transformation and by 6.4 to 6.6 in a log transformation. This is a standard procedure in a biological study that either uses Euclidean distance as a measure of dissimilarity or a Bray-Curtis index (Anderson 2001, Clarke & Warwick 2001, Legendre & Gallagh-

er 2001, Chapman & Bulleri 2003, Clarke *et al*. 2006, De Cáceres *et al*. 2013). Consequently, obtaining strictly precise and detailed quantitative information about the covers or abundances of every taxa is probably less important when we transform the data to reduce the effects of species with high abundances.

CONCLUSIONS

Marine hard bottom habitats and communities have a high heterogeneity at different spatial scales (Blanchard & Bourget 1999, Bulleri & Chapman 2004, Fraschetti *et al*. 2005) and it is often difficult to obtain robust scientific information from them. The almost invariable occurrence of interactions between factors at different scales makes it difficult to extract the ecological patterns governing the functioning of these interesting habitats (Underwood & Chapman 1996, 1998, Chapman 2003, Chapman & Bulleri 2003, Bulleri & Chapman 2004, Pister 2009), and sometimes, the differences between the communities are never evident (Chapman 2006, Pister 2009). Every methodology used in the study of hard benthic communities needs to make several decisions: which part of the community to study, the variable used to quantify it, the size and number of sampling units, etc. There are advantages and disadvantages associated with every sampling method (Benedetti-Cecchi *et al*. 1996), and the most appropriate sampling methodologies depend on the objectives of the study. The use of the methodology described here (Time-constrained sampling for a large sampling unit) in a comparative study depends on the maintenance of a homogeneous sampling effort, which, in its turn, depends on the intervention of the same previously trained sampling team. Thus, the specific data obtained by this methodology could be difficult to compare with other studies, although the global trends and conclusions could be comparable. Nevertheless, this methodology presents a good cost-benefit balance: it provides a good representation of the diversity of hard bottom communities and the costs (both in time and economic resources) for sampling and sorting are lower and the quantification of taxa allows their relative abundance to influence the results. This methodological line, already used by some researchers, could be an acceptable alternative that could thus be chosen for studying marine hard bottom communities and would be worth exploring further.

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Received on March 3, 2020
Accepted on January 25, 2021
Associate editor: A Migné