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Adapting metabarcoding-based benthic biomonitoring into routine marine ecological status assessment networks

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ABSTRACT

The use of genomic approaches to assist with biodiversity estimations is an alternative to traditional biomonitoring, which is very time-consuming and costly. In response to the high demand for quick community descriptions, DNA metabarcoding can simultaneously assign taxonomy to hundreds of samples rapidly and at low cost. However, the technique has not routinely been incorporated into biomonitoring network programs yet. Here, we applied DNA metabarcoding methodologies at stations within the monitoring network of the Basque Water Agency, the competent authority for the application of the European Water Framework Directive in this region. We characterized the benthic macroinvertebrate communities from 18 estuarine and coastal sediment samples using morphology and metabarcoding-based taxonomic identification and evaluated the performance of several versions of the AZTI's Marine Biotic Index (AMBI). Although metabarcoding detected 112 taxa against the 206 taxa identified through morphology, we showed that metabarcoding leads to similar biomonitoring conclusions compared with traditional techniques. Using the abundance and biomass of those taxa detected from morphological methodologies, we found a significant positive correlation with the number of reads obtained with metabarcoding approaches. The metabarcoding-based index derived from read counts, gAMBI, and the morphology-based index derived from individuals' biomass, (B)AMBI, showed the best correlation and revealed excellent agreement at determining the ecological status of the stations analyzed. We calculated that, for the analysis of the 51 stations included in the Basque monitoring network, metabarcoding was 55% less costly and 72% less time consuming. The results of our study are relevant to policy makers and researchers in the field of ecological assessment and will contribute to the quick implementation of DNA metabarcoding to intensive monitoring programs.

1. Introduction

Molecular taxonomy offers novel perspectives for environmental monitoring (Keck et al., 2017) and can improve the assessment of the marine environment (Bik et al., 2012; Dafforn et al., 2014; Goldberg et al., 2015). Since Taberlet et al. (2012) introduced the term 'DNA metabarcoding', this technique has been evaluated to assess biodiversity for ecosystem conservation purposes (Ji et al., 2013; Thomsen and Willerslev, 2015; Deiner et al., 2017). DNA metabarcoding results in the high-throughput identification of species by amplifying a short fragment of total DNA extracted from an environmental sample (i.e. soil, water, sediment). This technique has been proven to be effective for assessing changes in community structure along a disturbance gradient (Chariton et al., 2015; Keeley et al., 2018; Stoeck et al., 2018), for early detection of invasive species (Pochon et al., 2015; Zaiko et al., 2015), or for accurately assessing the marine benthic and planktonic diversity (Leray and Knowlton, 2015; Pearman and Irigoien, 2015; Chain et al., 2016; Wangensteen and Turon, 2016), among others. Yet, despite the documented potential of metabarcoding for monitoring, the gap between the scientific literature and management plans suggests that these applications need to be more effectively translated for policy making.

Monitoring programs evaluating environmental quality changes over time usually rely on the assessment of biological indicators using morphological taxonomy. However, these evaluations are time-consuming, expensive, and demand high-level taxonomic expertise (Yu et al., 2012). Therefore, depending on the number of sites and samples analyzed, evaluating environmental quality could require months until biomonitoring conclusions are obtained. The European Water Framework Directive (WFD, 2000/60/EC) and Marine Strategy Framework

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Directive (MSFD, 2008/56/EC) have highlighted the need to develop faster, more cost-efficient and reliable tools for the assessment of the marine environmental status (Heiskanen et al., 2016). Metabarcoding can improve such assessments through simultaneously assessing taxonomic composition of hundreds of samples at relatively low cost (Stein et al., 2014; van Dijk et al., 2014) and, in just a few weeks (Ji et al., 2013). Thus, the technique can greatly increase the number of sites or samples that can be monitored and the frequency of the assessments.

During the past decade, significant efforts have been made to test, validate and review the potential of metabarcoding to accurately monitor marine biological communities (Bucklin et al., 2016; Danovaro et al., 2016; Goldberg et al., 2016). Some downsides that could prevent the successful application of metabarcoding in environmental biomonitoring have been highlighted. For example, PCR biases can prevent the detection of all taxa within a sample (Deagle et al., 2014), and the lack of standardized sample processing strategies can strongly affect species detection success (Creer et al., 2016). Also, metabarcoding presents certain limitations in providing accurate estimations of organism abundance or biomass (Elbrecht and Leese, 2015). Despite the recognized limitations of the technique, several studies have demonstrated that metabarcoding is able to reliably characterize indicators of marine environmental status such as phytoplankton (Visco et al., 2015) or benthic macroinvertebrates (Lejzerowicz et al., 2015; Aylagas et al., 2016a). Further, metabarcoding has permitted the identification of new indicators of stress that are being neglected by international directives due to difficulties in their identification using morphological characters, such as bacteria or microbial eukaryotes (Aylagas et al., 2017; Keeley et al., 2018). Moreover, in the past ten years the cost of metabarcoding has greatly reduced (van Dijk et al., 2014) and it is anticipated that its contribution to a faster evaluation of the marine environment will be significant (Darling et al., 2017). For instance, an increasing number of studies have emphasized the potential of metabarcoding to improve resolution and cost-effectiveness for marine environmental management (Borja et al., 2016b; Darling et al., 2017). However, the application of metabarcoding in long-term marine monitoring programs is currently lacking.

This paper aims to test the potential of metabarcoding to determine marine ecological status using the Basque estuarine and coastal monitoring network program (Borja et al., 2016a) as a case study. First, we performed morphological and metabarcoding-based characterization of the macrobenthic community and then compared the morphology-based biotic index AMBI (AZTI's Marine Biotic Index; Borja et al., 2000) and the metabarcoding-based biotic index gAMBI (genomic AMBI; Aylagas et al., 2014). The aim is to evaluate the accuracy of gAMBI in providing environmental status assessments compared to those provided by AMBI. Further, through an exhaustive budget analysis using this particular case of study, we analyzed the capacity of metabarcoding to increase the speed and reduce costs of determining the environmental status of the locations under study in comparison to traditional monitoring techniques.

2. Materials and methods

2.1. Sampling and morphology-based taxonomic assignment

Samples were collected from 18 locations included in the Basque estuarine and coastal monitoring network (Borja et al., 2016a) (Fig. 1). These stations were selected as part of the sampling efforts done on a regular basis within the monitoring network of the Basque Water Agency in order to cover all type of sediments and the potential anthropogenic pressures present along the Basque coast (details of sediment type and depth are provided in Table S1). Sublittoral stations were sampled using a van-Veen grab (0.07–0.1 m^2), whereas the intertidal stations were sampled using a spade covering an area of 0.25 m^2. Four sediment samples were collected from each location and sieved on site using a sieve with a 1 mm mesh. Three of the samples were stored in formalin at room temperature and one in 96% ethanol (5:1 v/v) at 4 °C. From the formalin stored samples macroinvertebrate specimens were counted and identified to the lowest possible taxonomic level, and biomass of each taxa was determined as ash-free dry weight, obtained by drying in an oven at 80 °C for 48 h and incinerating at 450 °C for 4 h in a muffle furnace. The ethanol stored samples were processed for metabarcoding-based taxonomic assignment as detailed below.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.ecolind.2018.07.044.

2.2. Metabarcoding-based taxonomic assignment

Sample processing and genomic DNA extraction from the ethanol preserved sediment samples were performed following the procedure detailed in Aylagas et al. (2016b). From the total extracted DNA, a 313 bp fragment of the mitochondrial cytochrome oxidase I (COI) gene was amplified using the degenerated metazoan universal primer pair mlCOIintF-dgHCO2198 (Leray et al., 2013) with overhang Illumina adapters as in Bourlat et al. (2016). The PCR profile consisted of an initial 3 min denaturation step at 98 °C; 27 cycles of 10 sec at 98 °C, 30 sec at 46 °C and 45 sec at 72 °C; and a final 5 min extension at 72 °C. Equimolar concentrations of each dual-indexed PCR product were pooled and sequenced on the Illumina MiSeq platform with 2 × 300 bp paired-end v3 chemistry. Sequences were demultiplexed using the Miseq Reporter version 2.4.60.8. Sequence analysis and taxonomic assignments were performed following the pipeline described in Aylagas and Rodríguez-Ezpeleta (2016).

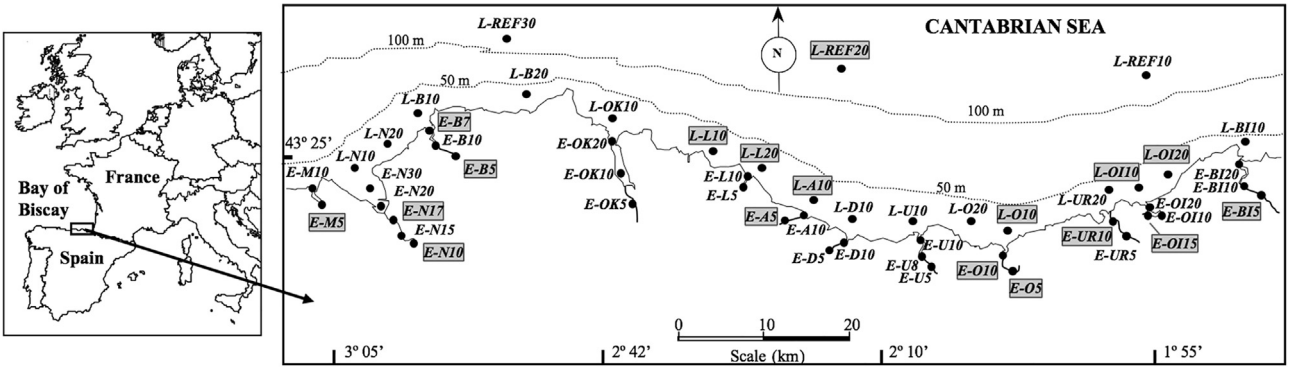

Fig. 1. Location of the 51 stations within the monitoring program network sampled in the Basque coast. Grey shaded locations were sampled within this study for morphology and metabarcoding-based macroinvertebrate taxonomic assignments and derived biotic indices. All locations were included to perform the analysis of cost and time required to calculate morphology and metabarcoding-based biotic indices within the monitoring program.