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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).

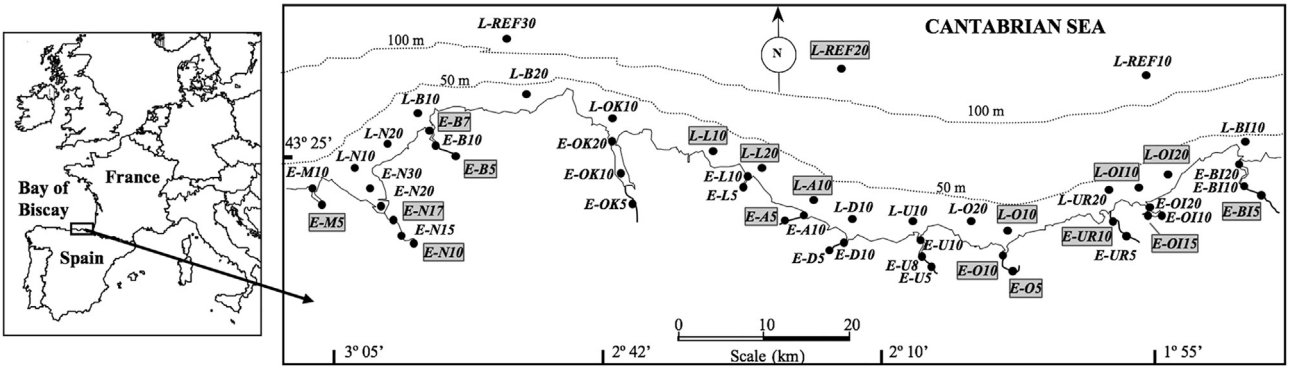

Fig. 1. Location of the 51 stations within the monitoring program network sampled in the Basque coast. Grey shaded locations were sampled within this study for morphology and metabarcoding-based macroinvertebrate taxonomic assignments and derived biotic indices. All locations were included to perform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

2.3. Biotic indices comparisons using morphology and metabarcoding-based taxonomic assignments

The lists of taxa obtained at each station using morphology and metabarcoding-based marine benthic macroinvertebrate taxonomic assignments were compared using an in-house script (see details in Aylagas et al., 2016a). Significant differences were tested using the Analysis of Molecular Variance (amova) implemented in mothur (Schloss, 2009). The amova used distance matrix constructed using Jacard (presence/absence) and Bray-Curtis (abundance) indices, which were obtained from both morphology and metabarcoding community tables. AMBI was calculated based on pollution tolerances of the species present, with tolerance being expressed categorically as one of five ecological groups (I: sensitive to pressure, II: indifferent, III: tolerant, IV: second order opportunist, and V: first order opportunist) using the AMBI 5.0 software (http://ambi.azti.es). Different versions of this biotic index were calculated: AMBI, (B)AMBI and (pa)AMBI (Warwick et al., 2010; Muxika et al., 2012) based on abundance, biomass and presence/absence of the morphologically identified specimens, respectively. Alternatively, the genetic versions of the index, gAMBI and (pa)gAMBI, were calculated using metabarcoding derived read counts and presence/absence for each identified taxon. The agreement between the disturbance classifications assigned to each site using traditional taxonomy (i.e. different versions of AMBI) and molecular taxonomy (i.e. different versions of gAMBI) was analyzed using a Kappa analysis (Cohen, 1960). The level of agreement is described using the ranges suggested by Monserud and Leemans (1992) for each value of Kappa: < 0.05, no agreement; 0.05–0.20, very poor; 0.20–0.40, poor; 0.40–0.55, fair; 0.55–0.70, good; 0.70–0.85, very good; 0.85–0.99, excellent and 1, perfect. The multivariate extension of AMBI, M-AMBI, which includes a combination of AMBI, richness and diversity (Muxika et al., 2007), was calculated at each site. This index uses reference conditions, which were set using historical data and modelling (Muxika et al., 2007). The boundaries between the different quality classes were established by intercalibration with other European indices (Borja et al., 2007). A genomic version of M-AMBI (named here M-gAMBI) was inferred using gAMBI and metabarcoding derived richness and diversity. The calculations were undertaken using the AMBI 5.0 software and the boundaries used were the same in the morphological and genomic indices. Further, the relationship between morphological identification derived abundances or biomass and metabarcoding identification derived read counts were assessed through a Pearsons's correlation coefficient.

2.4. Relative cost of morphology vs. metabarcoding-based environmental status assessment

The comparison between the effort necessary for environmental biomonitoring using morphology and metabarcoding was evaluated through the analysis of: (i) the time required to obtain the final results (referred as "waiting time"); and (ii) the economic cost including reagents, sequencing and personnel (referred as "economical cost") to process samples to an endpoint where all versions of AMBI and gAMBI are obtained (see Table S2 for details regarding the budget and time required for each part of the process. This table can be adapted by other researchers according to their specific investigations and used to estimate their budgets). Comparisons were made based on the information gathered from the taxonomic experts identifying the species for this research, based on morphology (cultural society INSUB, Spain) and from AZTI for the costs associated to metabarcoding-based taxonomic identification. In the case of the traditionally used AMBI, the estimated time to calculate the index for one sample is about 6.5 h (assuming experts in the classification of the specimens). For both methodologies, labor costs were set to 44 € per person per hour. Costs associated to field sampling were excluded as they are the same for both methodologies. The sequencing cost was calculated assuming multiplexing up to 384 samples on an Illumina MiSeq run and assuming that no samples from other projects could be used to complete the run.

Table 1
Number of quality-filtered reads, percentage of reads corresponding to macroinvertebrates and number of macroinvertebrate taxa identified per station using metabarcoding. (*) Samples processed in triplicate.

Sample	Number of quality filtered reads	Percentage of quality filtered reads that matched to macroinvertebrates	Number of macroinvertebrate taxa
E-A5	85,627	40.98	37
E-B5	74,326	61.54	3
E-B7	84,417	50.75	10
E-BI5	34,048	5.49	13
E-M5	75,409	21.86	14
E-N10	72,198	0.57	6
E-N17	51,535	55.22	10
E-O10	80,335	8.02	13
E-O5	57,912	58.72	11
E-OI15	60,319	0.85	4
E-OI15R*	70,053	0.72	5
E-OI15R2*	50,191	0.70	3
E-UR10	74,123	66.46	3
E-UR10R*	69,737	66.19	3
E-UR10R2*	68,360	66.00	3
L-A10	50,933	1.41	15
L-L10	83,842	0	0
L-L20	62,595	3.34	8
L-O10	85,539	12.25	5
L-OI10	59,260	18.10	10
L-OI20	73,589	0.20	2
L-REF20	88,878	62.55	21

3. Results and discussion

3.1. Morphology and metabarcoding-based benthic macroinvertebrate taxonomic assignments differ

From the total high-quality reads obtained using metabarcoding, 31% were assigned to macroinvertebrates (see Table 1) accounting for a total of 112 different taxa. Of the remaining reads, 29% could not be assigned to any metazoan phylum using the customized BOLD database (Aylagas and Rodríguez-Ezpeleta, 2016), whilst 36% were assigned to non-metazoans, and 4% remained unclassified (Fig. S1). From the 206 morphologically identified macroinvertebrate taxa, about 20% (range across stations from 0 to 66.6%) were detected using metabarcoding (see Tables S3 and S4 for the list of taxa detected by each method). Whilst the community composition of each sample was significantly different ($p < 0.001$) when using morphology or metabarcoding-based species identification (Fig. 2), the same comparison between morphological replicates did not reveal significant differences ($p > 0.05$). The discrepancies detected between morphology and metabarcoding-based taxonomy are the result of several factors inherent to the metabarcoding approach that influence detection/non-detection of certain species (Cowart et al., 2015; Lejzerowicz et al., 2015; Zimmermann et al., 2015).

For example, environmental DNA extraction procedure can amplify the differences between morphology and metabarcoding-based taxonomic compositions. Here, we followed a protocol that extracts DNA from a subsample of homogenized material thus avoiding the animal sorting step. This procedure requires certain sample manipulation, such as decanting (see details in Aylagas et al., 2016b), but reduces the processing time and allows for the technique to be standardized, which is a crucial requisite for the adoption of metabarcoding into routine monitoring network programs (Creer et al., 2016; Goodwin et al., 2017). However, this strategy can favor the detection of larger specimens, while smaller organisms can remain undetected (Elbrecht and

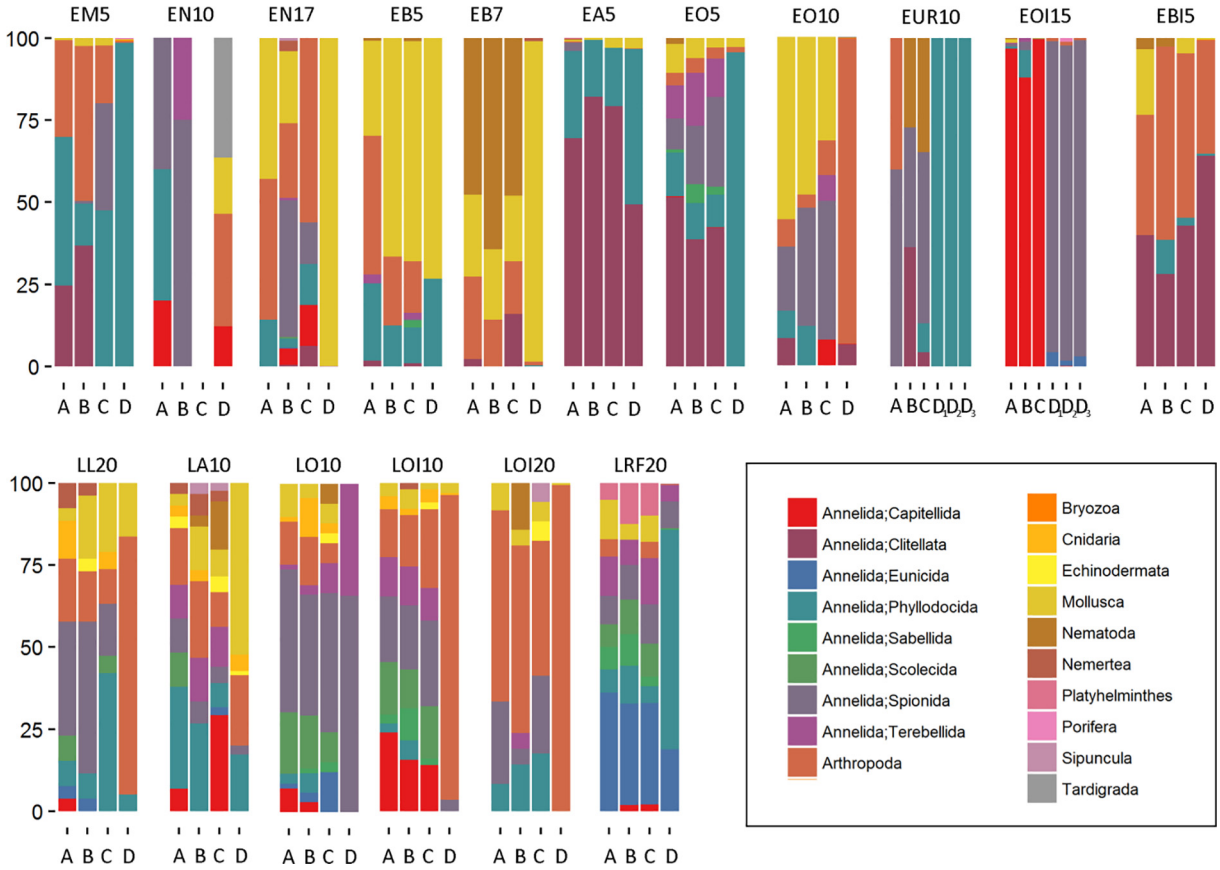

Fig. 2. Proportions of taxa identified using morphology (A, B, C) and metabarcoding (D) for each station. The taxonomy is presented at the order level for Annelida, and at the phylum level for remaining groups. Metabarcoding replicates performed on two of the samples are shown as D_1, D_2 and D_3.

Leese, 2015). Furthermore, when using homogenized samples, amplification of non-targeted taxa is likely to occur. This can explain the high percentage of quality filtered reads assigned to non-targeted taxa such as other metazoans (non macroinvertebrates), fungi or protists found here. This pattern has previously been observed (Lejzerowicz et al., 2015), but its consequences were not discussed. Thus, the different sample processing used here for morphology and metabarcoding-based taxonomic identification could have intensified the discrepancies in the taxonomic composition between both techniques.

Also, the marker gene and the primers used for its amplification can affect the taxonomic description of the environmental sample (Elbrecht and Leese, 2017). Here, we used a universal primer targeting a short COI DNA barcode (Leray et al., 2013) that has been applied to biodiversity assessments in different marine environments (Leray and Knowlton, 2015; Zaiko et al., 2015). Previous studies targeting the COI gene (Dowle et al., 2015; Elbrecht and Leese, 2015; Aylagas et al., 2016a) showed that metabarcoding typically recovers about 80% or even less of the taxa present in the sample. Lejzerowicz et al. (2015) targeted the V4 region of the 18S rRNA gene and found important differences between morphological and metabarcoding-based analyses at low taxonomic levels but reported a high congruence between morphology and metabarcoding for the most abundant taxa. Therefore, PCR bias caused by variable primer-template mismatches of individual species (Piñol et al., 2015) together with the taxonomic resolution of the targeted marker gene can compromise the comprehensive biological characterization of environmental samples using metabarcoding.

Another weakness of metabarcoding-based taxonomy is the dependence upon a reference database to translate the DNA reads into species names (Taberlet et al., 2012). The accuracy of the taxonomic assignments relies on the quality of the information contained in such database (Keck et al., 2017). In order to ensure the deposition of good quality barcode sequences, the collaboration between molecular ecologists and taxonomists is essential. The investment required for the generation of DNA barcodes accounts to about 5 € per specimen (see Stein et al., 2014), which together with the time required to identify each specimen and the high species diversity, makes it virtually impossible to build the perfect DNA reference database (Ji et al., 2013). We have previously suggested that at least for the most frequent and abundant species in monitoring networks, barcodes must be present in the repository to obtain accurate taxonomic inferences suitable for ecological status assessments (Aylagas et al., 2014). In an effort to increase the DNA barcode repository of the macroinvertebrate species within the Basque estuarine and coastal monitoring network, we have deposited 129 COI sequences available in “BCAS project” at BOLD (http://www.boldsystems.org).

The abovementioned drawbacks could hamper the application of metabarcoding in routine biomonitoring. Yet, accurate taxonomic inferences could be achievable by addressing the following procedures. For example, extracting DNA from bulk samples of previously isolated organisms (Chariton et al., 2010; Creer et al., 2010; Shokralla et al., 2015; Aylagas et al., 2016a) may ensure that DNA from all specimens in a sample is extracted. Doing *in silico* analysis to develop the best primers for the target community (Elbrecht and Leese, 2017) would enhance the performance of the primers used and, including different marker genes in the same study could improve the taxa detection success (Bucklin et al., 2016). Also, increasing the coverage of the reference databases is expected to enhance the taxonomic assignment of a great number of sequences (Beng et al., 2016). Although these practices will likely enable metabarcoding to be more reliably used in biodiversity taxonomic assignments they will require a significant economical and time investment, which contrasts with the current limited budgets existing for biomonitoring (Bourlat et al., 2013).

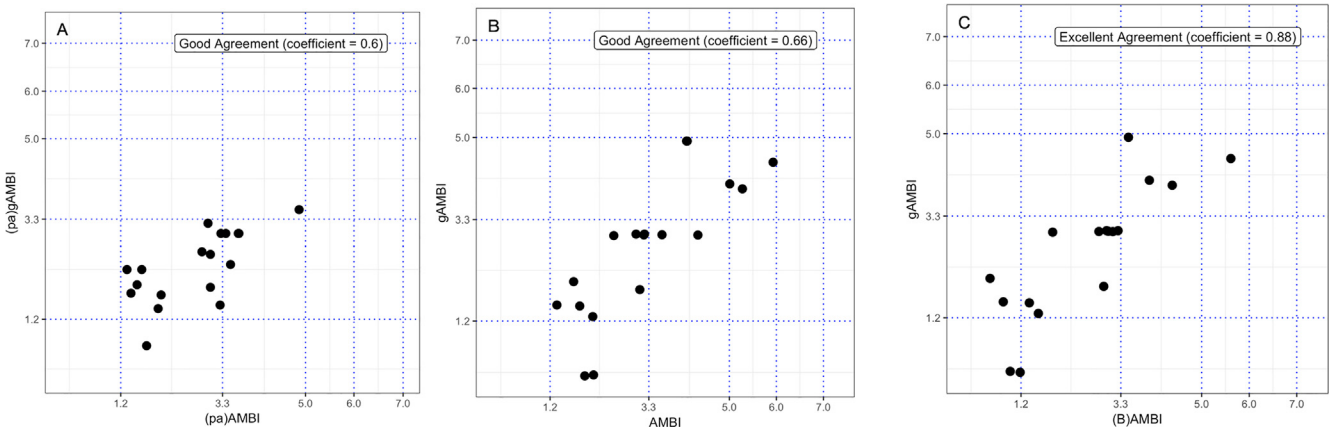

Fig. 3. Correspondence between (pa)AMBI and (pa)gAMBI (A), AMBI and gAMBI (B), and (B)AMBI and gAMBI (C). Vertical and horizontal lines are depicted using threshold values to discriminate disturbance classes: undisturbed [AMBI ≤ 1.2], slightly disturbed [1.2 < AMBI ≤ 3.3], moderately disturbed [3.3 < AMBI ≤ 5], heavily disturbed [5 < AMBI ≤ 6] and extremely disturbed [AMBI > 6]. The agreement between the morphology and metabarcoding-based biotic indices inferred from the Kappa analysis is shown.

3.2. Morphology and metabarcoding-derived biotic indices provide comparable marine benthic quality assessment

The different taxonomic composition obtained with morphology and metabarcoding could suggest that molecular approaches provide unreliable management conclusions. However, metabarcoding can detect changes in community composition across a disturbance gradient (Dafforn et al., 2014; Chariton et al., 2015; Stoeck et al., 2018), suggesting the potential of the technique to be applied to environmental quality assessment. Here, we found good correlations ($r^2 > 0.5$) between the metabarcoding-derived biotic indices (gAMBI and (pa)gAMBI) and the different versions of the morphology-based biotic index (AMBI) inferred from the same stations (Fig. 3). The Kappa analysis revealed "good" agreement between (pa)AMBI and (pa)gAMBI and, between AMBI and gAMBI and, "excellent" agreement between (B)AMBI and gAMBI, where 14 out of the 18 stations were classified under the same ecological status category. Similar comparisons performed between all versions of AMBI calculated from the three replicate samples resulted in very good correlations ($r^2 > 0.75$), although some discrepancies in the ecological status categories were observed (Fig. S2). The findings of this study suggest that metabarcoding provides accurate management conclusions within the current monitoring programs. However, adapting the boundaries to the different indices, the agreement between the morphology and metabarcoding-based biotic indices could be improved (Borja et al., 2007).

The metabarcoding derived read count version, gAMBI, could provide a more comprehensive evaluation of the ecological status than using (pa)gAMBI (based on presence/absence). However, due to the difficulties in the estimation of species abundances or biomass using PCR-based approaches (Piñol et al., 2015), the inclusion of metabarcoding in biomonitoring relying only on presence/absence metrics has been suggested (Yu et al., 2012; Dowle et al., 2015). The recently developed multi-trophic Metabarcoding Biotic Index (mt-MBI, Keeley et al., 2018) characterized key molecular ecological groups of three taxonomic groups (foraminifera, bacteria, and general eukaryotes) based on presence/absence. Also for gAMBI, only the presence/absence version has been validated (Aylagas et al., 2014; Heiskanen et al., 2016) but it reduces the importance of dominant taxa to the overall community (Warwick et al., 2010; Muxika et al., 2012) and might produce erroneous monitoring conclusions. Thus, finding correlation between sequence data and species abundance has been the focus of a number of studies (Thomsen et al., 2012; Goldberg et al., 2013; Evans et al., 2016). In general, low associations between macroinvertebrate species abundance or biomass and read number have been obtained (Dowle et al., 2015; Elbrecht and Leese, 2015). Here, using the abundance and biomass of those taxa detected from both morphology and metabarcoding methodologies, we found a significant positive correlation with number of reads using metabarcoding (Pearson's $r = 0.84$, $p < 0.0001$ for abundance, and Pearsons's $r = 0.8$, $p < 0.0001$ for biomass; Fig. 4). These findings should be strongly considered for biomonitoring as they show the potential of metabarcoding to provide species quantification and represent a step forward in the implementation of metabarcoding in management.

3.3. A cost efficient high-throughput biomonitoring in practice

The morphology and metabarcoding-based biotic indices calculated in the present study yielded similar biomonitoring conclusions indicating that both methodologies could assist in detecting changes in the ecological status of the community analyzed. The differences come from the economic costs and time required to process the samples (see Table S2). Metabarcoding showed reduced costs when several samples are simultaneously analyzed for calculating the genomic AMBI (Fig. 5).

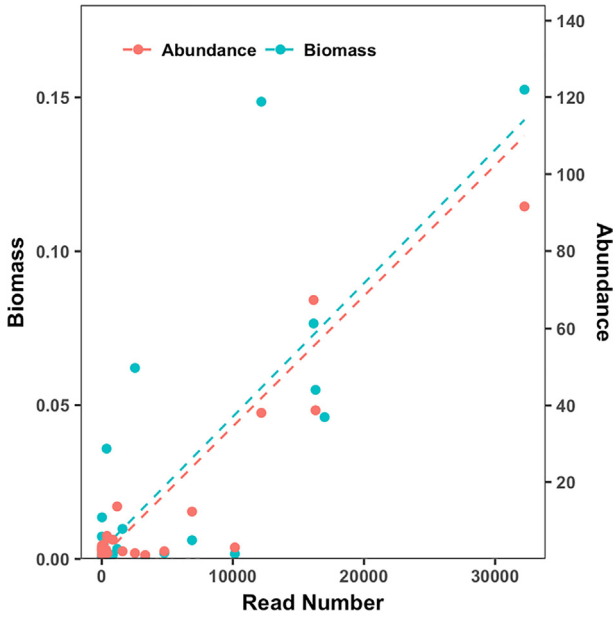

Fig. 4. Relationships between the abundance and biomass of each taxon at each site determined using morphology, and the number of reads generated for each taxon by metabarcoding.

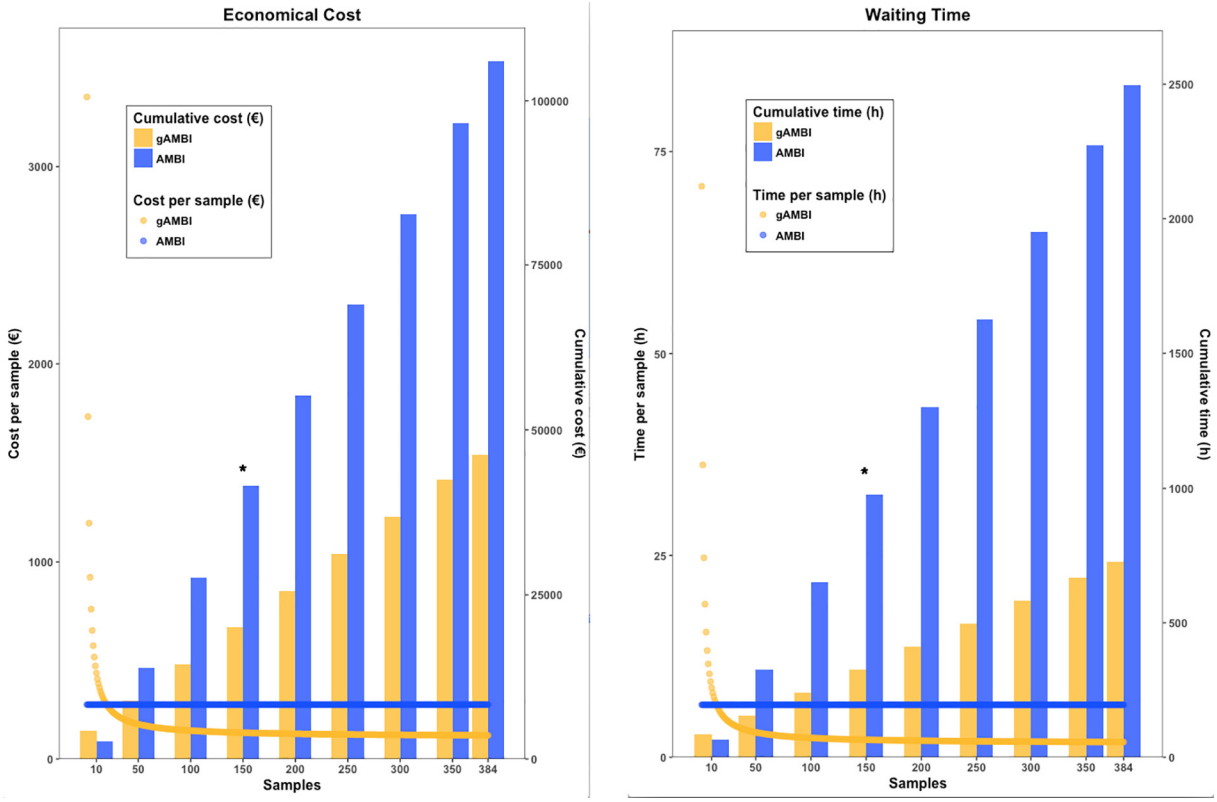

Fig. 5. Economical cost and waiting time associated to morphology and metabarcoding-based biotic indices calculation. The figure shows how the cost and time required to obtain metabarcoding-based biotic indices decrease with the number of samples analyzed. In contrast, regardless of the number of processed samples, the cost and time required to obtain traditional biotic indices remain constant. (*) Costs and time estimates to the annual effort of the entire Basque network program (153 samples).

These gains in cost-efficiency made possible by metabarcoding can greatly benefit large-scale biomonitoring programs (Ji et al., 2013). DNA methodologies for species detection have previously been proven to be a cheaper alternative for a single targeted taxa (Biggs et al., 2015; Sigsgaard et al., 2015; Smart et al., 2016). Here, we have extended the analysis to a community level and integrated it into a monitoring program.

We estimated that the Basque monitoring network (Borja et al., 2016a) spends 44,000 € annually evaluating the macroinvertebrate ecological status of 51 estuarine and coastal locations in triplicate (a total of 153 samples) (see Fig. 1). Providing biomonitoring results using morphological identification requires about 1,000 h for all locations (excluding field sampling time), which in practice represents 6 months. Based on current cost estimates, metabarcoding permits processing of the same number of samples in less time (about 280 h) and at lower costs (20,000 €) (Fig. 5). According to these calculations, in the current case of study, the use of metabarcoding represents a cost-saving of 55% and a time-saving of 72% compared to the traditional approach. Due to limited conservation budgets, monitoring programs are decreasing sampling frequency, reducing resolution and impeding comprehensive evaluations of the ecosystem integrity (Keck et al., 2017). The cheaper and faster alternative of metabarcoding offers an opportunity to increase the spatial and temporal resolution of biomonitoring programs.

3.4. Moving towards the inclusion of metabarcoding in routine marine monitoring

The WFD and MSFD directives, and the US EPA currently consider multivariate AMBI (M-AMBI) as a common approach to assess benthic conditions (Borja et al., 2009; Pelletier et al., 2018). The validation of a genomic-based M-AMBI (M-gAMBI) will favor the implementation of a

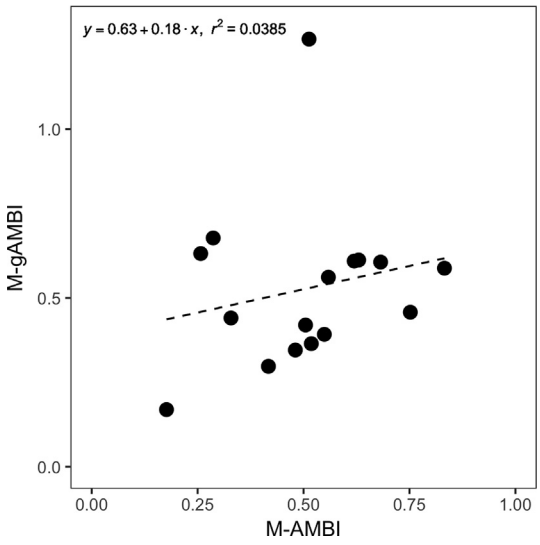

Fig. 6. Relationship between M-AMBI and M-gAMBI.

common metabarcoding tool for such assessments worldwide. Here, we found non-significant correlations between M-AMBI and M-gAMBI (Pearson's r = 0.19, p > 0.05, Fig. 6). Although comparable metabarcoding and morphology-based diversity metrics were obtained (no significant differences between genomic and morphology-based macroinvertebrate richness (p > 0.05) and, between genomic and morphology-based diversity (p > 0.05)), at some stations morphology detected more species than metabarcoding. This could explain the difficulty in the calculation of a reliable M-gAMBI. On the other hand, the assessment of the ecological quality status using M-gAMBI would

require previous classification of water bodies and typologies, together with the definition of their associated reference conditions, as done for M-AMBI (Borja et al., 2012). To establish such parameters, historical data, expert judgement and multivariate analysis is required. Therefore, further improvements in the multivariate version of gAMBI needs to be undertaken before its implementation in routine monitoring programs worldwide.

While further improvements are being implemented, we encourage the prompt inclusion of metabarcoding into environmental directives to make a productive use of the technique. For instance, gAMBI has been recently considered as a promising indicator for the MSFD by achieving a high score in the evaluation of the indicator fitness based on a series of quality criteria (Heiskanen et al., 2016). These criteria include scientific basis, ecosystem relevance, responsiveness to pressures, possibility to set targets, early warning capacity, concreteness, cost-efficiency, and existing and on-going data. Thus, to make an accurate and efficient biomonitoring routine, we suggest the use of metabarcoding in extensive monitoring programs and the sporadic coexistence of morphology and metabarcoding-based biodiversity assessments, as recently advocated by Hering et al. (2018). Performing periodic simultaneous large-scale traditional and metabarcoding-based monitoring will increase the number of comparisons between all versions of AMBI and gAMBI. Subsequently, these comparisons will allow the degree of incongruence between the two approaches to be confidently assigned. For future applications of gAMBI, we advocate the analysis of metabarcoding samples in triplicate as recommended by previous studies for measuring benthic impacts associated with marine activities such as aquaculture or oil and gas extraction platforms (Pawlowski et al., 2016; Laroche et al., 2018; Stoeck et al., 2018).

The process can be optimized through the collaboration of laboratories that routinely use this indicator in their local or national assessments. We propose the performance of the laboratory work at each institution following standardized procedures (i.e. applying the same DNA extraction protocol and consistency in the choice of primers). The samples could subsequently be combined in the same sequencing pool, followed by the simultaneous analysis of the raw data. In this scenario, even processing one individual sample following the present protocol and combining it on a flow cell in addition to other projects will represent a more cost-efficient practice than using the morphology-based approach. Due to some limitations regarding the total number of samples that can be multiplexed in a single MiSeq run, this number must be taken into consideration when designing the experiments. Once laboratories agree to combine efforts for sequencing and for the simultaneous analysis of data, results will be comparable over time and space, costs will be reduced and speed increased. To further improve the cost efficiency of gAMBI, future studies should test the viability of calculating the index from environmental DNA extracted from low amounts of sediment (e.g. 2–5 gr) using DNA extraction protocols applied in other studies (Pawlowski et al., 2016; Stoeck et al., 2018; Laroche et al., 2018). If suitable, this procedure will significantly reduce the cost and time in large-scale marine monitoring programs.

Metabarcoding-based monitoring could also provide a more comprehensive characterization of the biological community than conventional approaches through the description of different trophic levels, from microbial assemblages to macro-organism communities (Drummond et al., 2015). Collection of data on this taxonomic scale offers a new opportunity to incorporate different trophic levels into biomonitoring so that results can capture very recent short-term changes in sediment condition (Caruso et al., 2015) as well as longer-term pressures (Deiner et al., 2017). In order to adapt multi-trophic biodiversity assessments in marine monitoring, Keeley et al. (2018) combined the response of bacteria and eukaryotes to stressors into a single biotic index, which correlated very well with a morphology-based Enrichment Stage index. The recently developed bacterial community-based biotic index (microgAMBI, Aylagas et al., 2017) was shown to be sensitive to different level of pressures in coastal and estuarine environments at different locations (Borja, 2018). Moving in this direction, we encourage the investigation of a multi-gene biotic index to be incorporated in marine monitoring as a future version of gAMBI. Finally, we also favor the improvement of the biotic indices based on the reads clustered regardless the taxonomy assigned to each read, as suggested by Steele et al. (2011). Thus, the development of new indicators for environmental monitoring based on a total biodiversity metabarcoding profile could provide a more comprehensive DNA-based marine benthic quality assessment which is not dependent on the classification of a defined set of species.

4. Conclusion

In this study, we applied a metabarcoding approach to samples within a marine monitoring network program. This was accomplished through the parallel comparison of morphology-based biotic indices (all versions of AMBI) and metabarcoding-based biotic indices (all versions of gAMBI) calculated from samples collected within the Basque estuarine and coastal monitoring network program. The calculation of gAMBI required the application of recently developed protocols for laboratory sample processing, sequence analysis and taxonomic assignments of the benthic macroinvertebrate community. For the calculation of AMBI, the taxonomic classification of species was performed based on morphological characteristics. We show that biomonitoring conclusions obtained via metabarcoding-based approaches were comparable to those drawn by traditional methodologies. In particular, the best performance was obtained between gAMBI and the biomass derived index (B)AMBI. By performing an exhaustive budget analysis, we suggest that metabarcoding is a more cost-efficient marine biomonitoring method than traditional approaches (two times cheaper and three times faster). Consequently, we favor the prompt inclusion of genomic biotic indices into routine marine monitoring network programs. Our results indicate that further improvements need to be addressed to establish a common genomic biotic index to assess benthic conditions worldwide. Thus, whilst gAMBI can be immediately applied in routine monitoring programs, we encourage the focusing of efforts in the development of a multivariate genomic biotic index (M-gAMBI) to be implemented in the different marine directives. The findings presented in this study will contribute to the acceleration of the decision-making process in marine environmental management. This will allow more intensive monitoring programs (in time or regarding number of samples) to be undertaken, which will improve the quality of the assessments.

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References

Aylagas, E., Borja, A., Rodríguez-Ezpeleta, N., 2014. Environmental status assessment using DNA metabarcoding: towards a genetics based Marine Biotic Index (gAMBI). PLoS ONE 9, e90529.

Aylagas, E., Borja, A., Irigoien, X., Rodríguez-Ezpeleta, N., 2016a. Benchmarking DNA

metabarcoding for biodiversity-based monitoring and assessment. Front. Mar. Sci. 3.
Aylagas, E., Mendibil, I., Borja, Á., Rodríguez-Ezpeleta, N., 2016b. Marine sediment sample pre-processing for macroinvertebrates metabarcoding: mechanical enrichment and homogenization. Front. Mar. Sci. 3.
Aylagas, E., Borja, A., Tangherlini, M., Dell'anno, A., Corinaldesi, C., Michell, C.T., Irigoien, X., Danovaro, R., Rodriguez-Ezpeleta, N., 2017. A bacterial community-based index to assess the ecological status of estuarine and coastal environments. Mar. Pollut. Bull. 114, 679–688.
Aylagas, E., Rodríguez-Ezpeleta, N., 2016. Analysis of Illumina MiSeq amplicon reads: application to benthic indices for environmental monitoring. In: Bourlat, S.J. (Ed.), Marine Genomics Methods and Protocols, Methods in Molecular Biology, vol. 1452, Springer, New York.
Beng, K.C., Tomlinson, K.W., Shen, X.H., Surget-Groba, Y., Hughes, A.C., Corlett, R.T., Slik, J.W., 2016. The utility of DNA metabarcoding for studying the response of arthropod diversity and composition to land-use change in the tropics. Sci. Rep. 6, 24965.
Biggs, J., Ewald, N., Valentini, A., Gaboriaud, C., Dejean, T., Griffiths, R.A., 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). Biol. Conserv. 183, 19–28.
Bik, H.M., Porazinska, D.L., Creer, S., Caporaso, J.G., Knight, R., Thomas, W.K., 2012. Sequencing our way towards understanding global eukaryotic biodiversity. Trends Ecol. Evol. 27, 233–243.
Borja, A., 2018. Testing the efficiency of a bacterial community-based index (microgAMBI) to assess distinct impact sources in six locations around the world. Ecol. Ind. 85, 594–602.
Borja, A., Chust, G., Rodriguez, J.G., Bald, J., Belzunce-Segarra, M.J., Franco, J., Garmendia, J.M., Larreta, J., Menchaca, I., Muxika, I., Solaun, O., Revilla, M., Uriarte, A., Valencia, V., Zorita, I., 2016a. 'The past is the future of the present': learning from long-time series of marine monitoring. Sci. Total Environ. 566–567, 698–711.
Borja, Á., Dauer, D.M., Grémare, A., 2012. The importance of setting targets and reference conditions in assessing marine ecosystem quality. Ecol. Ind. 12, 1–7.
Borja, A., Franco, J., Perez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within european estuarine and coastal environments. Mar. Pollut. Bull. 40, 12.
Borja, A., Josefson, A.B., Miles, A., Muxika, I., Olsgard, F., Phillips, G., Rodríguez, J.G., Rygg, B., 2007. An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. Mar. Pollut. Bull. 55, 42–52.
Borja, A., Muxika, I., Rodríguez, J.G., 2009. Paradigmatic responses of marine benthic communities to different anthropogenic pressures, using M-AMBI, within the European Water Framework Directive. Mar. Ecol. 30, 214–227.
Borja, A., Elliott, M., Andersen, J.H., Berg, T., Carstensen, J., Halpern, B.S., Heiskanen, A.-S., Korpinen, S., Lowndes, J.S.S., Martin, G., Rodriguez-Ezpeleta, N., 2016b. Overview of integrative assessment of marine systems: the ecosystem approach in practice. Front. Mar. Sci. 3.
Bourlat, S.J., Borja, A., Gilbert, J., Taylor, M.I., Davies, N., Weisberg, S.B., Griffith, J.F., Lettieri, T., Field, D., Benzie, J., Glockner, F.O., Rodriguez-Ezpeleta, N., Faith, D.P., Bean, T.P., Obst, M., 2013. Genomics in marine monitoring: new opportunities for assessing marine health status. Mar. Pollut. Bull. 74, 19–31.
Bourlat, S.J., Haenel, Q., Finnman, J., Leray, M., 2016. Preparation of amplicon libraries for metabarcoding of marine eukaryotes using Illumina MiSeq: the dual-PCR method. In: Bourlat, S.J. (Ed.), Marine Genomics Methods and Protocols, Methods in Molecular Biology, vol. 1452, Springer, New York.
Bucklin, A., Lindeque, P.K., Rodriguez-Ezpeleta, N., Albaina, A., Lehtiniemi, M., 2016. Metabarcoding of marine zooplankton: prospects, progress and pitfalls. J. Plankton Res. 38, 393–400.
Caruso, G., La Ferla, R., Azzaro, M., Zoppini, A., Marino, G., Petochi, T., Corinaldesi, C., Leonardi, M., Zaccone, R., Fonda Umani, S., Caroppo, C., Monticelli, L., Azzaro, F., Decembrini, F., Maimone, G., Cavallo, R.A., Stabili, L., Hristova Todorova, N., Karamfilov V, K., Rastelli, E., Cappello, S., Acquaviva, M.I., Narracci, M., De Angelis, R., Del Negro, P., Latini, M., Danovaro, R., 2015. Microbial assemblages for environmental quality assessment, Knowledge, gaps and usefulness in the European Marine Strategy Framework Directive. Crit. Rev. Microbiol. 1–22.
Chain, F.J.J., Brown, E.A., Macisaac, H.J., Cristescu, M.E., Cowie, R., 2016. Metabarcoding reveals strong spatial structure and temporal turnover of zooplankton communities among marine and freshwater ports. Divers. Distrib. 22, 493–504.
Chariton, A.A., Court, L.N., Hartley, D.M., Colloff, M.J., Hardy, C.M., 2010. Ecological assessment of estuarine sediments by pyrosequencing eukaryotic ribosomal DNA. Front. Ecol. Environ. 8, 233–238.
Chariton, A.A., Stephenson, S., Morgan, M.J., Steven, A.D., Colloff, M.J., Court, L.N., Hardy, C.M., 2015. Metabarcoding of benthic eukaryote communities predicts the ecological condition of estuaries. Environ. Pollut. 203, 165–174.
Cohen, J., 1960. A coefficient of agreement for nominal scales. Educ. Psychol. Meas. 20, 37–46.
Cowart, D.A., Pinheiro, M., Mouchel, O., Maguer, M., Grall, J., Mine, J., Arnaud-Haond, S., 2015. Metabarcoding is powerful yet still blind: a comparative analysis of morphological and molecular surveys of seagrass communities. PLoS ONE 10, e0117562.
Creer, S., Fonseca, V.G., Porazinska, D.L., Giblin-Davis, R.M., Sung, W., Power, D.M., Packer, M., Carvalho, G.R., Blaxter, M.L., Lambshead, P.J., Thomas, W.K., 2010. Ultrasequencing of the meiofaunal biosphere: practice, pitfalls and promises. Mol. Ecol. 19 (Suppl 1), 4–20.
Creer, S., Deiner, K., Frey, S., Porazinska, D., Taberlet, P., Thomas, W.K., Potter, C., Bik, H.M., Freckleton, R., 2016. The ecologist's field guide to sequence-based identification of biodiversity. Methods Ecol. Evol. 7, 1008–1018.
Dafforn, K.A., Baird, D.J., Chariton, A.A., Sun, M.Y., Brown, M.V., Simpson, S.L., Kelaher, B.P., Johnston, E.L., 2014. Faster, higher and stronger? The pros and cons of molecular faunal data for assessing ecosystem condition. Adv. Ecol. Res. 51.
Danovaro, R., Carugati, L., Berzano, M., Cahill, A.E., Carvalho, S., Chenuil, A., Corinaldesi, C., Cristina, S., David, R., Dell'anno, A., Dzhembekova, N., Garcés, E., Gasol, J., Goela, P., Feral, J.-P., Ferrera, I., Forster, R., Kurekin, A.A., Rastelli, E., Marinova, V., Miller, P.I., Moncheva, S., Newton, A., Pearman, J., Pitois, S., Renè, A., Rodriguez-Ezpeleta, N., Saggiomo, V., Simis, S.G.H., Stefanova, K., Wilson, C., Lo Martire, M., Greco, S., Cochrane, S., Borja, A., 2016. Implementing and innovating marine monitoring approaches for assessing marine environmental status. Front. Mar. Sci. 3.
Darling, J.A., Galil, B.S., Carvalho, G.R., Rius, M., Viard, F., Piraino, S., 2017. Recommendations for developing and applying genetic tools to assess and manage biological invasions in marine ecosystems. Marine Policy 85, 54–64.
Deagle, B.E., Jarman, S.N., Coissac, E., Pompanon, F., Taberlet, P., 2014. DNA metabarcoding and the cytochrome c oxidase subunit I marker: not a perfect match. Biol. Lett. 10.
Deiner, K., Bik, H.M., Machler, E., Seymour, M., Lacoursiere-Roussel, A., Altermatt, F., Creer, S., Bista, I., Lodge, D.M., De Vere, N., Pfrender, M.E., Bernatchez, L., 2017. Environmental DNA metabarcoding: transforming how we survey animal and plant communities. Mol. Ecol. 26, 5872–5895.
Dowle, E.J., Pochon, X., Banks, J., Shearer, K., Wood, S.A., 2015. Targeted gene enrichment and high throughput sequencing for environmental biomonitoring: a case study using freshwater macroinvertebrates. Mol. Ecol. Resour. 16, 1240–1254.
Drummond, A.J., Newcomb, R.D., Buckley, T.R., Xie, D., Dopheide, A., Potter, B.C., Heled, J., Ross, H.A., Tooman, L., Grosser, S., Park, D., Demetras, N.J., Stevens, M.I., Russell, J.C., Anderson, S.H., Carter, A., Nelson, N., 2015. Evaluating a multigene environmental DNA approach for biodiversity assessment. Gigascience 4, 46.
Elbrecht, V., Leese, F., 2015. Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass-sequence relationships with an innovative metabarcoding protocol. PLoS ONE 10, e0130324.
Elbrecht, V., Leese, F., 2017. Validation and development of COI metabarcoding primers for freshwater macroinvertebrate bioassessment. Front. Environ. Sci. 5.
Evans, N.T., Olds, B.P., Renshaw, M.A., Turner, C.R., Li, Y., Jerde, C.L., Mahon, A.R., Pfrender, M.E., Lamberti, G.A., Lodge, D.M., 2016. Quantification of mesocosm fish and amphibian species diversity via environmental DNA metabarcoding. Mol. Ecol. Resour. 16, 29–41.
Goldberg, C.S., Sepulveda, A., Ray, A., Baumgardt, J., Waits, L.P., 2013. Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*). Freshwater Sci. 32, 792–800.
Goldberg, C.S., Strickler, K.M., Pilliod, D.S., 2015. Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. Biol. Conserv. 183, 1–3.
Goldberg, C.S., Turner, C.R., Deiner, K., Klymus, K.E., Thomsen, P.F., Murphy, M.A., Spear, S.F., Mckee, A., Oyler-Mccance, S.J., Cornman, R.S., Laramie, M.B., Mahon, A.R., Lance, R.F., Pilliod, D.S., Strickler, K.M., Waits, L.P., Fremier, A.K., Takahara, T., Herder, J.E., Taberlet, P., Gilbert, M., 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods Ecol. Evol. 7, 1299–1307.
Goodwin, K.D., Thompson, L.R., Duarte, B., Kahlke, T., Thompson, A.R., Marques, J.C., Caçador, I., 2017. DNA sequencing as a tool to monitor marine ecological status. Front. Mar. Sci. 4.
Heiskanen, A.-S., Berg, T., Uusitalo, L., Teixeira, H., Bruhn, A., Krause-Jensen, D., Lynam, C.P., Rossberg, A.G., Korpinen, S., Uyarra, M.C., Borja, A., 2016. Biodiversity in marine ecosystems—european developments toward robust assessments. Front. Mar. Sci. 3.
Hering, D., Borja, A., Jones, J.I., Pont, D., Boets, P., Bouchez, A., Bruce, K., Drakare, S., Hänfling, B., Kahlert, M., Leese, F., Meissner, K., Mergen, P., Reyjol, Y., Segurado, P., Vogler, A., Kelly, M., 2018. Implementation options for DNA-based identification into ecological status assessment under the European Water Framework Directive. Water Res. 138, 192–205.
Ji, Y., Ashton, L., Pedley, S.M., Edwards, D.P., Tang, Y., Nakamura, A., Kitching, R., Dolman, P.M., Woodcock, P., Edwards, F.A., Larsen, T.H., Hsu, W.W., Benedick, S., Hamer, K.C., Wilcove, D.S., Bruce, C., Wang, X., Levi, T., Lott, M., Emerson, B.C., Yu, D.W., 2013. Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. Ecol. Lett. 16, 1245–1257.
Keck, F., Vasselon, V., Tapolczai, K., Rimet, F., Bouchez, A., 2017. Freshwater biomonitoring in the information age. Front. Ecol. Environ. 15, 266–274.
Keeley, N.B., Wood, S.A., Pochon, X., 2018. Development and preliminary validation of a multi-trophic metabarcoding biotic index for monitoring benthic organic enrichment. Ecol. Ind. 85, 1044–1057.
Laroche, O., Wood, S.A., Tremblay, L.A., Ellis, J.I., Lear, G., Pochon, X., 2018. A cross-taxa study using environmental DNA/RNA metabarcoding to measure biological impacts of offshore oil and gas drilling and production operations. Mar. Pollut. Bull. 127, 97–107.
Lejzerowicz, F., Esling, P., Pillet, L., Wilding, T.A., Black, K.D., Pawlowski, J., 2015. High-throughput sequencing and morphology perform equally well for benthic monitoring of marine ecosystems. Sci. Rep. 5, 13932.
Leray, M., Knowlton, N., 2015. DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. PNAS 112, 2076–2081.
Leray, M., Yang, Y.J., Meyer, P.C., Mills, C.S., Agudelo, N., Ranwez, V., Boehm, T.J., Machida, J.R., 2013. New versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Front. Zool. 10.
Monserud, R.A., Leemans, R., 1992. Comparing global vegetation maps with the Kappa statistic. Ecol. Model. 62, 275–293.
Muxika, I., Borja, A., Bald, J., 2007. Using historical data, expert judgement and

multivariate analysis in assessing reference conditions and benthic ecological status, according to the European Water Framework Directive. Mar. Pollut. Bull. 55, 16–29.
Muxika, I., Somerfield, P.J., Borja, Á., Warwick, R.M., 2012. Assessing proposed modifications to the AZTI marine biotic index (AMBI), using biomass and production. Ecol. Ind. 12, 96–104.
Pawlowski, J., Lejzerowicz, F., Apotheloz-Perret-Gentila, L., Visco, J.A., Esling, P., 2016. Protist metabarcoding and environmental biomonitoring: time for change. Eur. J. Protistology 55, 12–25.
Pearman, J.K., Irigoien, X., 2015. Assessment of zooplankton community composition along a depth profile in the central red sea. PLoS One 10 e0133487.
Pelletier, M.C., Gillett, D.J., Hfamilton, A., Grayson, T., Hansen, V., Leppo, E.W., Weisberg, S.B., Borja, A., 2018. Adaptation and application of multivariate AMBI (M-AMBI) in US coastal waters. Ecol. Ind. 89, 818–827.
Piñol, J., Mir, G., Gomez-Polo, P., Agusti, N., 2015. Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative metabarcoding of arthropods. Mol. Ecol. Resour. 15, 819–830.
Pochon, X., Zaiko, A., Hopkins, G.A., Banks, J.C., Wood, S.A., 2015. Early detection of eukaryotic communities from marine biofilm using high-throughput sequencing: an assessment of different sampling devices. Biofouling 31, 241–251.
Schloss, P.D., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.
Shokralla, S., Porter, T.M., Gibson, J.F., Dobosz, R., Janzen, D.H., Hallwachs, W., Golding, G.B., Hajibabaei, M., 2015. Massively parallel multiplex DNA sequencing for specimen identification using an Illumina MiSeq platform. Sci. Rep. 5, 9687.
Sigsgaard, E.E., Carl, H., Møller, P.R., Thomsen, P.F., 2015. Monitoring the near-extinct European weather loach in Denmark based on environmental DNA from water samples. Biol. Conserv. 183, 46–52.
Smart, A.S., Weeks, A.R., Van Rooyen, A.R., Moore, A., Mccarthy, M.A., Tingley, R., Yoccoz, N., 2016. Assessing the cost-efficiency of environmental DNA sampling. Methods Ecol. Evol. 7, 1291–1298.
Steele, J.A., Countway, P.D., Xia, L., Vigil, P.D., Beman, J.M., Kim, D.Y., Chow, C.E.T., Sachdeva, R., Jones, A.C., Schwalbach, M.S., Rose, J.M., Hewson, I., Patel, A., Sun, F., Caron, D.A., Fuhrman, J.A., 2011. Marine bacterial, archaeal and protistan association networks reveal ecological linkages. ISME J. 5, 1414–1425.
Stein, E.D., Martinez, M.C., Stiles, S., Miller, P.E., Zakharov, E.V., 2014. Is DNA barcoding actually cheaper and faster than traditional morphological methods: results from a survey of freshwater bioassessment efforts in the United States? PLoS ONE 9, e95525.
Stoeck, T., Kochems, R., Forster, D., Lejzerowicz, F., Pawlowski, J., 2018. Metabarcoding of benthic ciliate communities shows high potential for environmental monitoring in salmon aquaculture. Ecol. Ind. 85, 153–164.
Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., Willerslev, E., 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. Mol. Ecol. 21, 2045–2050.
Thomsen, P.F., Kielgast, J., Iversen, L.L., Wiuf, C., Rasmussen, M., Gilbert, M.T., Orlando, L., Willerslev, E., 2012. Monitoring endangered freshwater biodiversity using environmental DNA. Mol. Ecol. 21, 2565–2573.
Thomsen, P.F., Willerslev, E., 2015. Environmental DNA – an emerging tool in conservation for monitoring past and present biodiversity. Biol. Conserv. 183, 4–18.
Van Dijk, E.L., Auger, H., Jaszczyszyn, Y., Thermes, C., 2014. Ten years of next-generation sequencing technology. Trends Gnet 30, 418–426.
Visco, J.A., Apotheloz-Perret-Gentil, L., Cordonier, A., Esling, P., Pillet, L., Pawlowski, J., 2015. Environmental monitoring: inferring the diatom index from next-generation sequencing data. Environ. Sci. Technol. 49, 7597–7605.
Wangensteen, O.S., Turon, X., 2016. Metabarcoding Techniques for Assessing Biodiversity of Marine Animal Forests. Springer International Publishing Switzerland 2016 1 S. Rossi (ed.), Marine Animal Forests.
Warwick, R.M., Robert Clarke, K., Somerfield, P.J., 2010. Exploring the marine biotic index (AMBI): variations on a theme by Ángel Borja. Mar. Pollut. Bull. 60, 554–559.
Yu, D.W., Ji, Y., Emerson, B.C., Wang, X., Ye, C., Yang, C., Ding, Z., 2012. Biodiversity soup: metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. Methods Ecol. Evol. 3, 613–623.
Zaiko, A., Samuiloviene, A., Ardura, A., Garcia-Vazquez, E., 2015. Metabarcoding approach for nonindigenous species surveillance in marine coastal waters. Mar. Pollut. Bull. 100, 53–59.
Zimmermann, J., Glockner, G., Jahn, R., Enke, N., Gemeinholzer, B., 2015. Metabarcoding vs. morphological identification to assess diatom diversity in environmental studies. Mol. Ecol. Resour. 15, 526–542.