



Research paper

Testing the efficiency of a bacterial community-based index (microgAMBI) to assess distinct impact sources in six locations around the world



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ABSTRACT

MicrogAMBI is a bacterial community-based index, recently developed to assess the ecological status of marine systems. The easy access to High Throughput Sequencing (HTS) technologies, allowing microgAMBI the quick taxonomic identification of bacterial assemblages, providing an early warning signal to assess impacts. MicrogAMBI was developed, applied to human pressures (organic and metal pollution), and validated, in estuaries and coasts of the Basque Country (northern Spain). Here, to test the efficiency of the index, six case studies have been selected, including different substrata and environments (sediment, water column, coral, and a sludge reactor), from polar to temperate and tropical locations. The pressures include a polluted sludge reactor, underground discharges, diffuse pollution, sedimentation, sewage outfalls and eutrophication, as well as two pristine areas, in some cases with spatial and temporal gradients. To calculate the index, the new taxa list includes 693 taxa. The list, being public, will allow to achieve a consensus in the assignment of taxa to ecological groups. This would facilitate the assessment of multiple human pressures, based upon bacteria composition. The study has demonstrated that microgAMBI has the potential to work reasonably well under very different environments and pressures. The index provides a single, clear and efficient way to establish the ecological status, based upon bacteria community composition, along spatial and temporal pressure gradients.

1. Introduction

Different legislation worldwide tends to perform integrative assessments of marine waters, including distinct ecosystem components (i.e. phytoplankton, macroalgae, macroinvertebrates, fishes, etc.) (Borja et al., 2008, 2016). After these authors, the most prominent global, regional or national legislation related to marine monitoring and assessment includes: (i) the United Nations Convention on Law of the Sea (UNCLOS, 1982), as the international basic legal framework that governs the uses of the oceans and seas; (ii) the Convention on Biological Diversity (CBD, 2000), as the international obligation to protect and use the resources of the marine environment sustainably; (iii) the Australian Oceans Policy (Commonwealth of Australia, 1999, 2006); (iv) the Canadian Oceans Act, and Oceans Strategy (Parsons, 2005); (v) the US Oceans Act and the Clean Water Act (Granek et al., 2005); (vi) the European Water Framework Directive (WFD; European Commission, 2000) and Marine Strategy Framework Directive (MSFD; European Commission, 2008); (vii) the South African National Water Act (www.dwaf.gov.za/documents/publications); (viii) the People's Republic of China laws on Water (1988/01/21) and Environmental Protection (1989/12/26).

Most of this legislation emphasises the assessment and achievement

of good quality status of aquatic systems, based upon the response of the abovementioned ecosystem components (i.e. phytoplankton, macroalgae, etc.) to different stressors (Ban and Alder, 2008; Nöges et al., 2016). The ability to monitor aquatic ecosystems is crucial to avoid adverse effects of human activities and pressures on marine communities, by implementing management measures (Halpern et al., 2008). However, to measure such effects, we need indicators, biotic indices or multimetric assessment methods, which have been developed by hundreds in past two decades, for different ecosystem components (Díaz et al., 2004; Birk et al., 2012; Borja et al., 2015). Therefore, among these components, and even though bacteria are in the basis of most biogeochemical cycles in the nature (Martiny et al., 2006), they have been ignored when developing assessment indices. Only very recently, Aylagas et al. (2017) have proposed a bacterial community-based index to assess the ecological status of estuarine and coastal environments.

The idea of the index is based upon the fast response of bacteria to environmental changes, both in degraded and recovery systems, and the concept, applicability and boundary conditions were established by Aylagas et al. (2017). In addition, the easy access to High Throughput Sequencing (HTS) technologies, allows the quick taxonomic identification of bacterial assemblages, providing an early warning signal to assess impacts in marine systems (Aylagas et al., 2017). To develop this

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bacterial biotic index, Aylagas et al. (2017) followed the strategy of the AZTI's Marine Biotic Index (AMBI; Borja et al., 2000), classifying 216 different taxa into two ecological groups (EG): EGI, as taxa not associated with pollution inputs (including sensitive and indifferent taxa), and EGIII, as taxa associated with disturbance or pollution inputs (including tolerant and opportunistic taxa). The authors named the index 'microAMBI', since it is based on the microbial assemblage composition (16S rRNA gene amplicon sequencing) and is inspired in AMBI.

MicroAMBI was developed, applied to human pressures (organic and metal pollution), and validated, in estuaries and coasts of the Basque Country (northern Spain). Taking into account the response of bacteria to different stressors, it is likely expected that microAMBI could be applied to other biogeographical areas worldwide; its application should be conditioned only by the assignation of new taxa to the ecological groups, in which microAMBI is based. Hence, the main objectives of this research are: (i) to increase the initial list of taxa assigned to the EGs, making it freely available; (ii) to explore the application of microAMBI to different areas across the globe, whenever possible across pressure gradients; and (iii) to assess its usefulness in relation to different impact sources.

2. Material and methods

2.1. Expanding the taxa list

To expand the initial taxa list, a review of the literature was done to select common bacteria taxa appearing in environmental studies. The taxa were assigned to EGIII based on the criteria set by Aylagas et al. (2017), related to their ecological role associated with pollution inputs and disturbance, including: (i) dominance in organic matter-enriched sediments; (ii) organic pollution response; (iii) dominant presence in anoxic methane-rich sediments; (iv) identification as nitrite oxidizer and related to nitrogen inputs; (v) presence in sulfide-rich wastewaters; (vi) presence in wastewater treatment plants; (vii) role in methanogenic degradation of alkanes; (viii) role in aromatic compounds biodegradation, including petroleum products pollution, as complex PAHs; and (ix) potential pathogens. The remainder taxa were assigned to EGI (e.g. aerobic taxa, taxa described as living in pristine systems, etc.), except when no clear indication exists in the literature. In these few cases, the taxa remained unassigned. The taxa list includes, for each taxon, the reference or web page link in which the assignation is based. This information is available as Supplementary Material (sheet 'taxalist').

2.2. Selecting the case studies

After completing the taxa list, a review of the literature was undertaken, looking for several case studies in which: (i) the abundance of bacteria can be extracted from the Supplementary Material of the studies, either in sediments or water column; (ii) they include different pollution sources, environmental problems and geographical setting; and (iii) some abiotic data or information from the authors exist to analyze the performance of microAMBI and/or interpret the results. A total of six cases (Fig. 1), totally independent from the taxa list, to avoid circularity, were selected for the analysis, as shown below.

2.2.1. Alaska

The study from McFarlin et al. (2017) aimed to identify the bacteria and functional genes able to degrade oil in a future oil exploitation in Chukchi Sea (Alaska), which has no human activity currently. They sampled 17 stations: 11 were sampled once and 6 stations twice, resulting in a total of 23 samples per water column depth. At each station, seawater samples were collected from the surface, the middle of the water column (20 m), and 2–3 m above the seafloor (near 40 m), on two separate surveys (August–September 2012). Environmental measurements consisted of temperature, salinity, chlorophyll-a, pheophytin, and macronutrients (phosphate, silicate, nitrate, nitrite,

ammonium, and dissolved inorganic nitrogen).

Bacterial community DNA was extracted from each sample and sequenced with 454-Pyrosequencing on the GS FLX Titanium platform using F563-577 and R907-924 primers to target the V4-V5 region on the 16S rRNA gene. The sequence length (350 bp) allowed McFarlin et al. (2017) to determine the Operational Taxonomic Units (OTUs) of bacteria present (down to the genus level when possible). The most abundant (> 1% of total abundance) OTUs in surface, middle and bottom seawater collected were obtained from Table S1, at the Supplementary Material of McFarlin et al. (2017).

As the area is not affected by human activity and is considered as pristine, it is expected that the ecological status, measured by microAMBI, should be high, and maybe some differences with depth can be detected.

2.2.2. China

The research undertaken by Wang et al. (2016) compared the effectiveness of three methods for the treatment of oil refinery waste activated sludge, in Beijing (China). The methods were a single phase anaerobic digestion, and two different pretreatment methods (acidogenic and methanogenic), and the authors expected a progressive effectiveness of the three methods, in degrading oil wastes.

The microbial communities were investigated by Illumina high-throughput sequencing. DNA was extracted and V3 and V4 regions of 16 S rDNA gene were selected for PCR, and two universal primers were selected for amplification. Data on bacteria (> 1% relative abundance), from the single-phase, hydrolytic-acidogenic and methanogenic reactors, at family or genus level, was obtained from Table 2 in Wang et al. (2016).

Taking into account the activity, it is expected that the sludge should be in less than good status and, if the different methods increase the efficiency degradation of the sludge, increasing microAMBI values should be recorded, since a dominance of opportunistic taxa should be found in the acidogenic and methanogenic methods.

2.2.3. Korea

The objective of Lee et al. (2017) was to investigate the responses of the coastal microbial community structure to tidal cycles, rainfall regime, and a submarine groundwater discharge at a coastal beach in Jeju Island (Korea). Water samples were taken at increasing distance of the discharge (close, 40 m and 300 m offshore), in September 2013, March and July 2014. Samples near to the discharge were taken at high tide, ebb tide, low tide, and flood tide. The other two locations were sampled only during low and high tide. Additionally, a sediment sample was collected near the discharge. Some abiotic variables were analyzed, such as salinity, dissolved oxygen, total phosphorous (TP), total nitrogen (TN) or silicate.

The microbial community was determined extracting the bulk genomic DNA, using 16S rRNA gene-based pyrosequencing, targeting V1 to V3 regions. The relative abundance (> 5% of total samples) of the representative OTUs was obtained from Table S2, in the Supplementary Material of Lee et al. (2017).

Taking into account the rainfall regime, tidal phase and distance to the discharge, it is expected that microAMBI values should be higher close to the coast, in ebb and low tide and in wet season, when the discharge is higher.

2.2.4. India

Verma et al. (2017) investigated the structure of the bacterial communities in deep-sea (1400–2000 m) sediment samples from the Bay of Bengal (two sites relatively close to polluted sites: Chennai and Cuddalore) and the Andaman Sea (Barren island, an uninhabited island with an active volcano and submarine activity). These authors hypothesized that the site-associated microbial diversity would depend on sea-sediment composition and anthropogenic impact. They analyzed the grain size, total organic carbon (TOC), TN and TP in the sediments.

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