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Challenges for implementing Next Generation Sequencing (NGS) of benthic macrofauna challenges, in the evaluation of marine environmental quality

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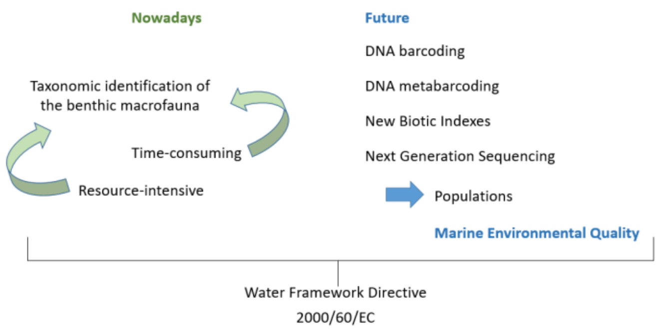
Graphical Abstract



Abstract

The Water Framework Directive 2000/60/EC regulates the environmental diagnosis of the marine ecosystem, including the evaluation of species of bioindicator macroinvertebrates present in the environment. To date, these types of determinations are carried out through the morphotaxonomic identification of the benthic macrofauna present in the samples and the calculation of associated biotic indexes, a process that is time-consuming and resource-intensive, being in some cases inaccurate due to the requirement of highly specialized human resources and the difficulty of correctly identifying certain species. In this respect, DNA barcoding techniques allow the reliable identification of organisms using DNA sequencing techniques and avoiding the disadvantages of morphotaxonomic identification. On the other hand, the recent development of New Generation DNA Sequencing techniques (NGS) has allowed the development of DNA metabarcoding, i.e. the characterization of populations of organisms present in a sample using genomic data. This paper shows the fundamental challenges to be

	overcome in order to establish a NGS sequencing-based assessment of the marine environmental quality.

Introduction

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Materials and Methods

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Results and Discussion

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Conclusions

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References

[1] European Commission. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. Official Journal 22 December 2000 L 327/1. European Commission, Brussels (2000).

[2] M. D. Subida, P. Drake, E. Jordana, B. Mavric, S. Pinedo, N. Simboura, J. Torres, F. Salas, Response of different biotic indices to gradients of organic enrichment in Mediterranean coastal waters: Implications of non-monotonic responses of diversity measures, *Ecol. Indic*. **19** 2012 106–117.

[3] J. C. Dauvin, T. Ruellet, Polychaete/amphipod ratio revisited, *Mar. Pollut. Bull*. **55** (2007) 215–224.

[4] N. Simboura, A. Zenetos, Benthic indicators to use in Ecological Quality classification of Mediterranean soft bottom marine ecosystems, including a new Biotic Index, *Mediterr. Mar. Sci*. **3** (2002) 77–111.

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

[6] A. Borja, J. Franco, V. Pérez, Marine Biotic Index to Establish the Ecological Quality of Soft-Bottom Benthos Within European Estuarine and Coastal Environments, *Mar. Pollut. Bull*. **40** (2000) 1100–1114.

[7] E. Aylagas, N. Rodríguez-Ezpeleta, Analysis of Illumina MiSeq Metabarcoding Data: Application to Benthic Indices for Environmental Monitoring, *Methods Mol. Biol*. **1452** (2016) 237–249.

[8] F. Lejzerowicz P. Esling, L. Pillet, T. A. Wilding, K. D. Black, J. Pawlowski, High-throughput sequencing and morphology perform equally well for benthic monitoring of marine ecosystems. *Sci. Rep*. **5** (2015) 13932.

[9] S. Pinedo, E. Jordana, Spain (Catalonia and Balearic Islands), in: A.C., Heiskanen, A.-S. (Eds.), Water Framework Directive Intercalibration Technical Report. Part 3: Coastal and Transitional waters. *JRC Scientific and Technical reports* (2007) 62–70.

[10] A. Dell'Anno, L. Carugati, C. Corinaldesi, G. Riccioni, R. Danovaro, Unveiling the Biodiversity of Deep-Sea Nematodes through Metabarcoding: Are We Ready to Bypass the Classical Taxonomy?, *PLoS One* **10** (2015) e0144928.

[11] M. Leray, N. Knowlton, Censusing marine eukaryotic diversity in the twenty-first century, *Philos. Trans. R. Soc. B Biol. Sci*. **371** (2016) 20150331.

[12] D. W. Yu, Y. Ji, B. C. Emerson, X. Wang, C. Ye, C. Yang, Z. Ding, Biodiversity soup: metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring, *Methods Ecol. Evol*. **3** (2012) 613–623.

[13] P. D. N. Hebert, A. Cywinska, S. L. Ball, J. R. DeWaard, Biological identifications through DNA barcodes, *Proc. Biol. Sci.* **270** (2003) 313–321.

[14] M. Hajibabaei, S. Shokralla, X. Zhou, G. A. C. Singer, D. J. Baird, Environmental Barcoding: A NextGeneration Sequencing Approach for Biomonitoring Applications Using River Benthos, *PLoS One* **6** (2011) e17497.

[15] E. Ferri, M. Barbuto, O. Bain, A. Galimberti, S. Uni, R. Guerrero, H. Ferté, C. Bandi, C. Martin, M. Casiraghi, Integrated taxonomy: traditional approach and DNA barcoding for the identification of filarioid worms and related parasites (Nematoda), *Front. Zool* **6** (2009) 1.

[16] S. E. Miller, DNA barcoding and the renaissance of taxonomy, *Proc. Natl. Acad. Sci*. **104** (2007) 4775–4776.

[17] C. J. Geraci, M. A. Al-Saffar, X. Zhou, DNA barcoding facilitates description of unknown faunas: a case study on Trichoptera in the headwaters of the Tigris River, Iraq, *North Am. Benthol. Soc*. **30** (2011) 163–173.

[18] R. Vivien, F. Lejzerowicz, J. Pawlowski, Next-Generation Sequencing of Aquatic Oligochaetes: Comparison of Experimental Communities, *PLoS One* **11** (2016) e0148644.

[19] M. Guardiola, M. J. Uriz, P. Taberlet, E. Coissac, O. S. Wangensteen, X. Turon, Deep-Sea, Deep-Sequencing: Metabarcoding Extracellular DNA from Sediments of Marine Canyons, *PLoS One* **10** (2015) e0139633.

[20] L. Yang, Z. Tan, D. Wang, L. Xue, M. Guan, T. Huang, R. Li, Species identification through mitochondrial rRNA genetic analysis, Sci. Rep. 4 (2014) 4089.

[21] R. J. Machida, M. Kweskin, N. Knowlton, PCR Primers for Metazoan Mitochondrial 12S Ribosomal DNA Sequences, *PLoS One* **7** (2012).

[22] A. Zhan, S. A. Bailey, D. D. Heath, H. J. Macisaac, Performance comparison of genetic markers for highthroughput sequencing-based biodiversity assessment in complex communities, *Mol. Ecol. Resour* **14** (2014) 1049–1059.

[23] S. F. Altschul, W. Gish, W. Miller, E. W. Myers, D. J. Lipman, Basic local alignment search tool, *J. Mol. Biol*. **215** (1990) 403–410.

[24] J. Ye, G. Coulouris, I. Zaretskaya, I. Cutcutache, S. Rozen T. L. Madden, Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction, *BMC Bioinformatics* **13** (2012) 134.

[25] V. Elbrecht, F. Leese, Can DNA-Based Ecosystem Assessments Quantify Species Abundance? Testing Primer Bias and Biomass—Sequence Relationships with an Innovative Metabarcoding Protocol, *PLoS One* **10** (2015) e0130324.

[26] S. Shokralla, T. M. Porter, J. F. Gibson, R. Dobosz, D. H. Janzen, W. Hallwachs, G. B. Golding, M. Hajibabaei, Massively parallel multiplex DNA sequencing for specimen identification using an Illumina MiSeq platform, *Sci. Rep*. **5** (2015) 9687.

[27] M. E. Carew, V. J. Pettigrove, L. Metzeling, A. A.Hoffmann. Environmental monitoring using next generation sequencing: rapid identification of macroinvertebrate bioindicator species. *Front Zool.* **10 (**2013) e45.

[28] J. F. Gibson, S. Shokralla, C. Curry, D. J. Baird, W. A. Monk, I. King I, M. Hajibabaei. Large-Scale Biomonitoring of Remote and Threatened Ecosystems via High-Throughput Sequencing. Fontaneto D, editor. *PLoS One* **10** (2016) e0138432.