DOI: http://doi.org/10.21698/simi.2020.ab21

DNA METABARCODING FOR ENVIRONMENTAL BIODIVERSITY EVALUATION

Catalina Stoica, Daniela Ionica, Lucian Ionescu, Daniel Mitru, Alina Roxana Banciu, Stefania Gheorghe, Irina Lucaciu, Mihai Nita-Lazar

National Research and Development Institute for Industrial Ecology – ECOIND, 71-73 Drumul Podu Dambovitei, 060652, Bucharest, catalina.stoica@incdecoind.ro, Romania

Keywords*: biodiversity, DNA metabarcoding, ecosystems, Water Framework Directive*

Introduction

The decline of biodiversity and aquatic ecosystem functioning is triggered by human activities which generate pollution, habitat degradation, flow modification, overexploitation and the spread of invasive species as well as climate change [1, 2]. Various legislation documents were globally adopted, including Water Framework Directive (WFD, 2000/60/EC) at European level, to reduce the degradation of aquatic ecosystems. The WFD has confirmed that the focal point of ecosystem integrity should be based on its biology rather than its chemical and physical characteristics. At the beginning of the 21th century, numerous assessment methods were proposed to focus on the biology as proposed in the WFD objectives outlined by the Member States of European Union. Most of the methods relied on morphological taxonomy and comprised similar workflow steps for aquatic bioassessment, such as: i) sampling of "biological quality elements" (BQE); ii) sorting; iii) identification morphology-based; iv) drawing-up the taxa list; v) assessment to an ecological quality class.

Environmental DNA (eDNA) metabarcoding, commonly named high-throughput amplicon sequencing [3] represents a novel method for biodiversity evaluation based on the genetic material analysis, collected from environmental samples as well as on micro- and macroorganisms [4]. The DNA metabarcoding approach is a viable alternative to morphological taxonomy methods and it is gradually integrated in the environmental biodiversity assessment studies. The eDNA metabarcoding workflow includes: i) isolation of eDNA from a single environmental component (water, sediment, soil) or bulk samples; ii) PCR amplification of a marker gene targeting biotic communities; iii) high-throughput sequencing of obtained amplicons; iv) quality filtering of identical sequences to obtain Individual Sequence Units (ISU); v) clustering of ISU into Operational Taxonomic Units (OTUs); vi) taxonomic assignment of OTUs and further data analysis-index calculation and quality assessment. The advantages of using eDNA metabarcoding over morphological taxonomy methods requires lower effort, high accuracy of species detection, the use of bulk species without a priori knowledge as well as early life stages, partially destroyed or fragmented species, increase comparability across geographic regions [5].

Moreover, eDNA metabarcoding could be integrated in monitoring studies across various ecosystems, such as freshwater, terrestrial, marine, estuarine (Table 1).

Table 1. eDNA metabarcoding applicability (adapted after Ruppert et al., 2019) [4]

Ecosystem type			
Freshwater	**Terrestrial**	**Marine**	**Estuarine**
Water / Sediment	**Soil**	**Water / Sediment**	**Water / Sediment**
Microbes	Fungi	Microbes	Microbes
Diatoms	Microbes	Plankton	Plankton
Plankton	Parasitoids	Fungi	Fungi
Protists	Arthropods	Protists	Protists
Plants	Plants	Diatoms	Diatoms
Fungi	Vertebrates	Plants	Plants
Invertebrates		Invertebrates	Invertebrates
Vertebrates		Vertebrates	Vertebrates

Overall, although this method has been developed and largely used in the last decade, there are several limitations that need to be consider in the future researches, such as: drawing-up standardized molecular protocols, harmonization of DNA preservation and isolation methods, selection of DNA indexes and PCR primers, the reference database for bioindicator taxa is incomplete, development of new biotic indices based on metabarcoding results as well as taxonomic assignment of OTUs and clustering.

Acknowledgment

This paper was financially supported by Nucleu Program, contract 20N/2019, project no. PN 19-04 02 01.

References

[1] Dudgeon, D., Arthington, A.H., Gessner, M.O, Kawabata, Z.I., Knowler, D.J., Leveque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J, Sullivan, C.A., 2006, Biol. Rev. 81, p.163-182.

[2] Leese, F., Bouchez, A., Abarenkov, K., Altermatt, F., Borja, A., Bruce, K., Ekrem, T., Ciampor, Jr., F., Ciamporova-Zatovicova, Z., Costa, F.O., Duarte, S., Elbrecht, V., Fontaneto, D., Franc, A., Geiger, M.F., Hering, D., Kahlert, M., Stroil, B.K., Kelly, M., Keskin, E., Liska, I., Mergen, P., Meissner, K., Pawlowski, J., Penev, L., reyjol, Y., Rotter, A., Steinke, D., van der Wal, B., Vitecek, S., Zimmermann, J., Weigand, A.M., 2018, Advan. Ecol. Research, 58, p. 63-99.

[3] Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C., Willerslev, E., 2012, Mol. Ecol.. 21, p. 2045-2050.

[4] Ruppert, K.M., Kline, R.J., Rahman, M.S., 2019, Glob. Ecol. Conserv., 17, e00547.

[5] Pawlowski, J., Kelly-Quinn, M., Altermatt, F., Apotheloz-Perret-Gentil, L., Beja, P., Boggero, A., Borja, A., Bouchez, A., Cordier, T., Domaizon, I., Joao Feio, M., Filipe, A.F., Fornaroli, R., Graf, W., Herder, J., van der Hoorn, B., Jones, J.I., Sagova-Mareckova, M., Moritz, C., Barquin, J., Piggott, J.J., Pinna, M., Rimet, F., Rinkevich, B., Sousa-Santos, C., Specchia, V., Trobajo, R., Vasselon, V., Vitecek, S., Zimmerman, J., Weigand, A., Leese, F., Kahlert, M., 2018, Sci. Total Environ., 637-638, p. 1295-1310.