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## ALTERNATIVE METHODS TO DETECT BIOLOGICAL COMMUNITIES IN FRESHWATER SYSTEMS

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

National Research and Development Institute for Industrial Ecology – ECOIND, 71-73 Drumul Podu Dambovitei, district 6, Bucharest, 060652, catalina.stoica@incdecoind.ro, Romania

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### **Introduction**

The Water Framework Directive (2000/60 / EC) (WFD) was adopted as a result of a global need to solve environmental issues and to archive environmental sustainability in a holistic approach taking into account the social requirements. The EU Member States have spent considerable resources to develop tools for river basin management plans.

The "ecological status" could be better characterized by the biologic communities' response to pressure factors (such as urban agglomeration, hydrotechnical works, domestic and industrial wastewater discharges etc), rather than the variability of physico-chemical parameters.

Even though WFD has been implemented as an important stimulant for the harmonization, classification and monitoring methods across Europe, there are still gaps in this approach.

Numerous studies published over the last five years focused on the difference between conventional methods of the biological communities morphological identification and alternative metabarcoding DNA methods, rapid and cost-effective methods for assessing the biodiversity of aquatic systems (Cordier et al 2019; Serrana et al 2019; Ruppert et al 2019; Pawlowski et al 2018; Hering et al 2018; Vasselon et al 2017; Elbrecht et al 2017).

### **Result and discussion**

The conventional methods involve the organisms sampling according to standard sampling methods followed by an estimation of taxonomic groups and their abundance. The collected data is used to calculate various indices compared with the reference values of indices considering the region (with little or no anthropogenic impact or historical data). Subsequently, the aquatic system is framed into ecological states (very good, good, moderate, poor, very poor).

#### *Alternative methods*

Alternative methods rely on the identification of biological models based on specific genes or DNA regions. The resulting PCR product after amplification is sequenced and compared to a reference library (Elbrecht et al 2017).

These alternative methods have been addressed because they allow a higher taxonomic resolution than morphological identification methods.

Additionally, the development of DNA sequencing technologies is a promising alternative to biodiversity monitoring, especially as these techniques can allow a rapid

taxonomy with high accuracy, cost-effective analysis, allowing the analysis of multiple samples simultaneously compared to conventional methods. Several limitations were described for conventional methods such as: a) time of analysis; b) higher costs; c) extensive and specific expertise of organisms groups (phytoplankton, phytobenthos and benthic invertebrates); d) difficulty to identify certain development stages of organism at lower level; e) the sorting of organisms is laborious and f) the determination keys for certain groups and in certain regions are unavailable (Lobo et al 2017; Serrana et al 2018).

The use of specific DNA sequences to identify biological communities can overcome the above mentioned problems. In other words, these alternative methods have the ability to fundamentally change the assessment of ecological status.

However, even these new alternative approaches presented some methodological limitations, such as: i) biomass estimation; ii) detection of rare species; iii) incomplete reference libraries (Majaneva et al 2018). It worth to be mentioned that an advantage of using molecular biology techniques is that of evaluating functional diversity based on gene extraction, fulfilling an objective of WFD that could not be adequately covered on the basis of conventional morphological identification methods.

Therefore, in order to achieve good environmental status for all aquatic systems by 2027, complementing and even replacing traditional sample processing methods must be a goal in the near future at national level, too.

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