Innovative methods and approaches for WFD: ideas to fill knowledge gaps in science and policy

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The Water Framework Directive – WFD (2000/60/EC) is the cornerstone of European Union water policy. Its main objective is to protect and enhance the status of aquatic ecosystems and promote sustainable water use across Europe. The WFD summarises much of the European experience of pollution, water quality and ecosystem management, and it represents a new and compressive way of source-to-sink thinking, where the primary goals are to achieve the desired quality of the water resources and to ensure that there is enough clean water for different applications. This way of thinking is widely accepted and the WFD is becoming the most substantial and ambitious piece of European environmental legislation to date. However, twenty years after the WFD has been introduced, achieving its objectives still remains a challenge, with 60% of EU surface waters failing the good ecological status in 2018. There is growing concern that the objective of good status in all EU waters (i.e. over 111.000 surface waters and over 13.000 groundwaters) by 2027 is a long way from being achieved in many countries. This paper discusses how science and water policy can incorporate innovations and new approaches and adapt to the newly identified challenges (e.g. biodiversity, climate change and springs ecosystems) facing European waters.

1. Introduction

The adoption of the Water Framework Directive - WFD (2000/ 60/ EC) completely changes the approach to water conservation, protection and management. The Directive represents a new comprehensive way of thinking about water bodies as a whole, in order to achieve adequate water quality and ensure satisfactory water qualities and quanitives for different needs (Herceg et al., 2018). The Water Framework Directive emphasizes the immense value of water and the need to manage it responsibly on the basis of a catchment approach, not only of inland waters, but also of transitional, coastal and groundwater, which are directly related (2000/60/ EC). The main purpose of the Water Framework Directive was to ensure the conservation of clean water and to improve the status of laden water bodies, as well as to contribute to reducing discharges of hazardous substances into water, promoting sustainable water use, protecting aquatic and terrestrial ecosystems and wetlands, and encouraging transboundary cooperation on water issues (Herceg et al., 2018). Over the past 20 years, the Directive has contributed to the development of new tenants and principles in the protection of environmental components and environmental legislation. It also encouraged research, the development of new, comparable methods for assessing the status of water bodies and better monitoring programs, and contributed to improving knowledge of the state of water in Europe, as well as water-threatening factors. However, the EU Member States, as well as the Accession States, have so far failed to achieve the primary objective of the Directive, which is to achieve at least a "good status" for all water bodies in their territories. In this paper authors summarize the output of a workshop, which aimed at identifying knowledge gaps in freshwater ecosystem monitoring related research and addressed the following research questions:

- What are the challenges and knowledge gaps in freshwater monitoring studies that are of outstanding importance?

Figure 1: *The main topics of the workshop (1)*

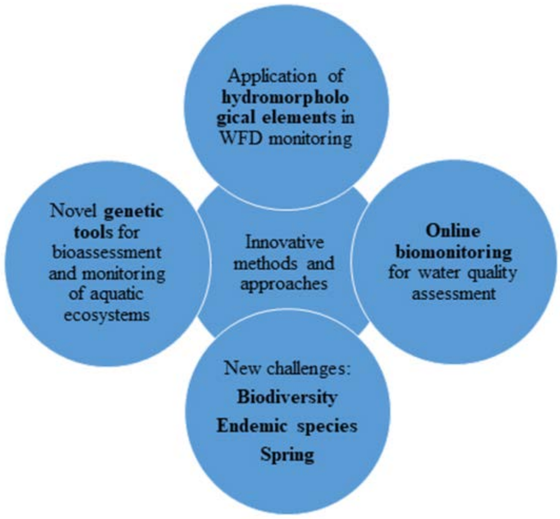

Figure 2: The main topics of the workshop (2)

- For the implementation of the innovative methods and approaches in management and integrated valuation of freshwaters and related policies?
- For future work in WFD research in general, where freshwater research can advance innovations research?
- For new challenges of WFD such as biodiversity and springs.

2. Materials and Methods

This paper is result of online workshop called "Innovative methodes and approaches for Water Framework Directive" organized by the Kompetenzzentrum Wasser Bodensee (Germany) in cooperation with the Adriatic Sea Basin Agency in Mostar, University of Mostar and relevant administrations authorities. This workshop has been organized as part of the regional project Danube Water Net, supported by the Baden Württemberg Stiftung. The workshop aimed to give an overview the Water Framework Directive, to establish contact with eminent European professional and scientific public and various stakeholders from the water sector, with the aim of networking and discussing new approaches and research gaps. In the following sections the main issues will be presented and complemented with suggestions of how to address these complex questions. Questions were selected that would address the key challenges and gaps in WFD implementation from their knowledge of the literature under the themes of the eWorkshop: (1) monitoring and assessment, (2) immplementation innovative methodes and approaches on management measures (3) future work of WFD and (4) new challanges of WFD as biodiversity and vulnerable habitats as springs.

3. Results

Altogether nine major topics were identified, with a number of challenges and knowledge gaps.

3.1. The Water Framework Directive: 20 Years On

Member States (as well as the Accession States) are obliged to provide for the river basin district, or part of the international river basin district located in their territories, an analysis of its characteristics, an overview of the impact of human activities on surface and groundwater status and an economic analysis of water use, and submit an implementation report (EC/2000/60). They are required to adopt national river basin management plans, laws, regulations and other administrative provisions necessary to harmonize legislation with this Directive (EC/2000/60). The Water Framework Directive sets out a clear implementation timetable, based on six-year management cycles. River basin management plans seek to ensure at least "good ecological status" of all waters, and the end of the first six-year management cycle, i.e. 2015, was set as the deadline for achieving it. The deadlines for achieving this status can be extended until 2027, if natural conditions require it. An extension is possible in the event of long-term recovery of the ecosystem after the applied restoration measures or a slow decrease in the nitrate concentration in groundwater. Implementation reports published to date have shown that, despite significant progress in achieving "good environmental status", success depends primarily on the ambitions of the Member States and the effective and measurable implementation of their plans (EEA report no. 7/2018). Compared to the previous six-year period (2009-2015), limited progress in improving the status of water bodies has been observed during the current implementation period (2015-2021). According to the Water Status Report published by the European Environment Agency in July 2018 (EEA report no. 7/2018), 74% of groundwater bodies achieved "good chemical status" and 89% "good quantitative status". Unlike groundwater, the WFD has unfortunately not achieved such good results for surface water bodies to date. By 2018, only 38% of them had "good chemical status", and 40% had "good ecological status" (or "good ecological potential" in the case of artificial and heavily modified water bodies). Among pollutants, mercury (Hg) was the most common cause of the lower environmental status of surface waters in the EU. Namely, out of over 111,000 surface water bodies in the EU, a total of 45,973 did not achieve "good chemical status" due to the presence of this heavy metal (EEA report no. 7/2018). In addition to mercury, "good chemical status" in the EU has not been achieved to date due to the presence of certain long-term organic pollutants (POPs) such as polybrominated diphenyl ether (pBDE), and the presence of some polycyclic aromatic hydrocarbons (PAHs). However, there have been improvements in the reduction of concentrations of certain heavy metals, such as cadmium (Cd), nickel (Ni) and lead (Pb) in surface waters, as well as improvements in pesticide content. Compared to the chemical status of waters during the first management cycle (until 2015), progress has been made in terms of monitoring coverage, as the number of water bodies with unknown status has been reduced from 39% to 16%. The chemical status of waters has improved in transitional and coastal waters, while only limited progress has been made in the chemical status of European streams and stagnant waters (EEA report no. 7/2018). Most European surface water bodies had not made significant progress in their status by 2019 compared to the previous implementation period (2009-2015), the European Commission concludes that progress in implementation is not satisfactory ((2007/60/EC), COM(2019)95). Possible reasons for limited achievements include: untimely identification of pressures on water bodies, lengthy development of effective policies and implementation of measures, and slowness of natural processes of auto-purification and restoration of aquatic ecosystems (EEA report no. 7/2018). Also, the poor results are partly due to improvements in monitoring programs and reporting. Namely, in recent years the standards for quality assessment have been raised, and monitoring has been extended to a larger number of water bodies in the EU, which were generally not in a satisfactory condition ("good or very good ecological status / potential") (EEA report no. 7/2018; 2007/60/EC; COM (2019) 95). The main pressures on aquatic ecosystems in the EU are defined as:

- hydro-morphological pressures and water extraction,
- diffuse sources of pollution - among which the most important are agriculture and atmospheric precipitation (mercury),
- point sources of pollution – especially industry and energy production.

Water as a resource in many parts of Europe remains under pressure, especially from economic activities, and EU environmental legislation faces new challenges. Namely, since the WFD was formally adopted in December 2000, a number of developmental changes have taken place, potentially affecting its implementation, such as the impact of climate change and the accompanying risks of floods and droughts, invasive species and pressures from the full spectrum of new pollutants present in the environment. New ways and principles of environmental management have been developed, such as the ecosystem services concept, and the UN has defined sustainable development goals, many of which are directly or indirectly related to water. In addition, new EU policies have been adopted, relating to biodiversity, renewable energy and flood risk management, which also affect the way water is managed. These changes will inevitably affect the tenants and principles set by the WFD, especially as 2027 approaches. Therefore, it is necessary to find opportunities for new innovative solutions and development of monitoring, management and water policy systems today in order to better implement the Directive.

3.1.1. Improving the system of monitoring and assessment of water bodies

One of the significant achievements of the WFD is certainly the development of an efficient and comparable system for monitoring the ecological status of waters across the EU (Birk et al., 2012).

However, the monitoring system developed within the WFD also has its weaknesses. Although biological quality elements are relatively well defined and developed, criteria for monitoring organic pollution, hydromorphological and morphological assessment are insufficient. Furthermore, there is very little data on the effects of multiple stressors on biological elements. Therefore, the principles of water protection and management based on a single stressor, while neglecting the interactions between multiple stressors, are unlikely to contribute to achieving the set management objectives and more efficient use of already limited resources in order to achieve them.

In the period that follows, the WFD must also adapt to the changes that our civilization is facing, such as global climate change. In its current form, the WFD has not yet developed an appropriate system for monitoring the status of temporary and occasional watercourses. Streams that periodically dry up are a natural occurrence in Mediterranean areas. Given that the number of these water bodies will increase due to global warming in the future, it is necessary to develop an appropriate monitoring and evaluation system, which the scientific community has been working on recently (for example COST action SMIRES - Science and Management of Intermittent Rivers and Ephemeral Streams).

The WFD monitoring and assessment system required by the WFD also requires immense resources, which also affects the implementation and scope of water monitoring programs. Therefore, the process of adapting the Directive should also take into account the opportunities provided by innovation and technology in monitoring processes, such as wider use of satellites and drones, DNA technol-

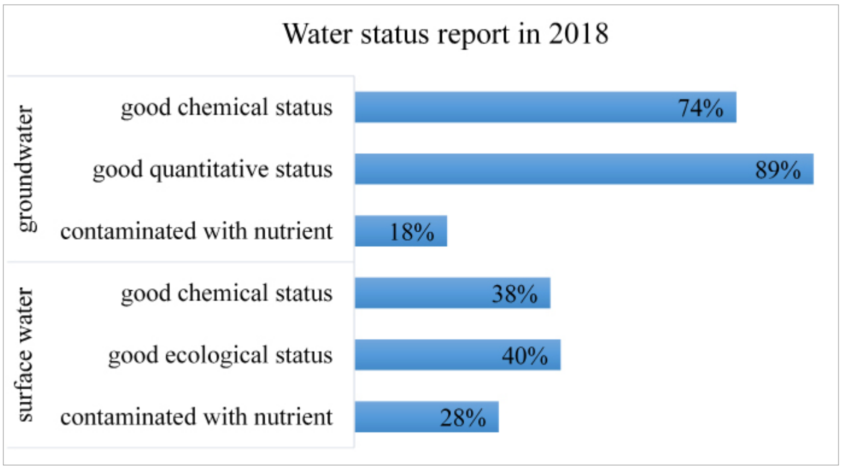

Figure 3: Water status report in 2018 for groundwater and surface water (EEA report no. 7/2018)

ogy (eDNA), online monitoring programs, etc. The cost of monitoring water quality on a precautionary basis is negligible in relation to the amounts that need to be invested for the implementation of restoration mitigation measures in case of deterioration of the ecological status.

3.1.2. Cross-sectoral cooperation and improvement of water management measures

In recent years, new perspectives on environmental management have been developed, which include the interaction of several components of the environment and offer solutions based on natural principles (Nature-Based Solutions – NBS). As there are no specific funds at EU level to finance the implementation of WFD measures, it is very important to link its implementation with activities and policies co-financed by EU funds or other international sources, such as ecosystem services or the UN sustainable development goals. The concept of ecosystem services ensures the healthy functioning of communities, and is in the centre of attention of the scientific and professional public of the 21st century. Today, encouraging development based on the assessment of ecosystem services is the basis for decision-making and policy at the international and European level and by offering a common platform between different fields of science and policy, ecosystem services today are the basis for sustainable development of local communities and society as a whole. As the services of aquatic ecosystems are numerous and immensely important, it is necessary to take them into account in water management processes.

3.1.3. Integrating water protection and management into other sectoral policies

Aware of the pressures on aquatic ecosystems, in 2012 the Commission adopted a Plan for the Protection of European Water Resources (COM (2012) 673) which emphasizes the need to put European water resources management in a broader perspective, taking into account all water users, and including the interaction between water and other resources, for example soil and energy. The European Water Resources Plan aims to manage water in a sustainable way, in all sectors that use water resources, such as industry, agriculture, tourism, urban development and energy production, to ensure a sufficient amount of quality water for present and future generations. Although this goal is already mentioned in the WFD, the European Water Resources Protection Plan facilitates its implementation by identifying remaining barriers and ways to overcome them. This approach to integrating water protection into other sectoral policies is necessary to achieve more concrete results in water protection. In the latest Water Status Report (EEA report no. 7/2018), the European Environment Agency highlights the impact of various sectors, in particular agriculture, forestry, energy production and urbanization, on the limited progress in achieving the WFD targets.
According to the Water Status Report, 28% of all surface waters and 18% of groundwater bodies are still contaminated with nutrients (EEA report no. 7/2018). Therefore, the agricultural sector is considered to be one of the most problematic in achieving the WFD targets. Agricultural production has multiple effects on the chemical and ecological status of water, contributing to: eutrophication by leaching nutrients from the soil, pesticide pollution, changes in the morphological characteristics of the riverbed and coastal zone by creating agricultural land, and water loss and hydromorphological significance of streams due to irrigation. Therefore, it is necessary to strengthen integration with the agricultural sector, and to better implement WFD measures through the CAP (Common Agricultural Policy). In addition, meeting the obligations arising from the WFD requires a stronger link between the water sector and biodiversity protection activities, plans and programs. Invasive species pose a serious threat to aquatic ecosystems in the EU, the preservation of indigenous aquatic organisms is a prerequisite for achieving at least "good" water status and their restoration.

3.1.4. The ecological mapping approach in the assessment of transformation river-sea ecosystems in the Danube Delta for development of WFD

The assessment of the state of the aquatic environment meets big challenges in coastal areas or in transition zones where the river and marine waters mix (Berlinskyi et al., 2005). The use of an ecological approach with the aspects of bioindication recommended by WFD together with modern statistical programs and mapping has already been tested for many fresh water bodies in different climatic zones (Barinova, 2017 a, b). However, the application of these approaches to assessing the state of marine ecosystems has not yet received widespread use due to the limited number of indicator species in sea communities (Barinova et al., 2016). According to results of Barinova et al. (2020-in press) microalgal research in the Ukrainian part of the Danube nearshore area more than half of the species were bioindicators with several ecological preferences: salinity, habitat, level of saprobity. Based on the Index of saprobity, Class 3 and 4 of water quality were identified for the studied area. The NT:PT ratio varied from 34:1 to 47:1 and from 27:1 to 72:1 in the surface and bottom layers, respectively, which indicate on the imbalance of the ecosystem by nitrogen. Based on ecological mapping and application of bioindicators the main trends in the transformation of river waters were showed. They identified the two pools of factors that form the development of the phytoplankton community: salinity and oxygen, on the one hand, and nutrients, on the other. The analysis of the maps demonstrated that dredging and dumping caused additional entrance of the nitrogen, phosphorus, and siliceous. The leading factor of the processes of production and destruction were the disbalance of nitrogen and phosphorus and the velocity of its recycling. Further application of the bioindication approach and the statistical mapping can develop the WFD implementations and help to assess the dynamic of trophic relationships in the dynamic transition ecosystem, such as the Danube nearshore area.

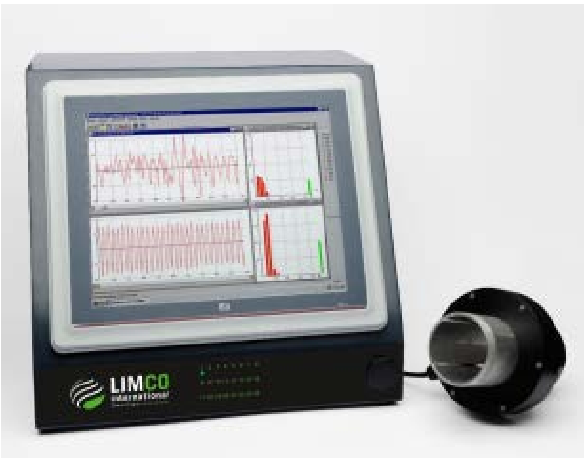

Figure 4: The Multispecies Freshwater Biomonitor (MFB)

3.2. Innovative methods and approaches

3.2.1 Online biomonitoring for water quality assessment in WFD monitoring

Online Biomonitoring systems have been developed for river biomonitoring of toxic pulses after the Sandoz accident 1986 in the river Rhine at the Swiss/German border. Along several large rivers in Europe streamside monitoring stations have been built an equipped with online animal biomonitors using Daphnia spp., mussels and/or fish as indicators for toxic stress in bypass systems. The aim was to protect the numerous drinking water supply facilities along the Rhine. These systems detected several acute toxic spills however they have the following disadvantages: the river water has to be filtered and warmed to 20 °C before it can be passed through the Daphniatoximeter. The availability of toxic substances is affected by these measures, hence the reality is not appropriately mirrored. The fishmonitors (mainly Leuciscus sp.) have been attacked by animal welfare organisations, leading to a stop of their usage. The mussel monitors, however, were not sensitive enough to indicate toxic pulses, partly due to the fact that mussels can close their shells for longer periods to avoid toxic stress (Gerhardt, 1999). These facts including the personnel efforts for maintenance and the decrease in acute toxic spills led to the closure of monitoring stations along the large rivers about ten years after their installation. However, meanwhile the 2nd generation of animal online biomonitors grew up, e.g. the Multispecies Freshwater Biomonitor© (MFB) with the following advantages:

- The water does not need to be filtered or pre-treated in any way, hence mirroring the real toxic load.
- All types of freshwater invertebrates can be used, hence the test species can be chosen according the the local conditions, thus improving the ecological relevance of the biomonitor results fort he receiving river ecosystem.
- Several different test species can be used simultaneously, which improves the estimation of the potential effects of a toxic pulse on the biocoenosis in the receiving stream, e.g. taking species from different trophic levels.
- Depending of the chosen test species, the maintenance efforts can be reduced enormously, e.g. by choosing particle feeders, which feed on the particles in the raw water and choosing long-living species, e.g. gammarids.
- Next to the application of protecting water quality of the raw water of drinking waterworks along the river Rhine a new application was born with these new ecologically oriented biomonitors: the use for monitoring ecosystem health and biodiuversity in surface waters.

The Multispecies Freshwater Biomonitor (MFB) (Gerhardt et al., 1994, 1998) is based on the non-optical quantitative recording principle of quadrupole impedance conversion (Gerhardt et al., 1994). An animal is placed in a cylindrical flow through chamber containing two pairs of stainless steel electrodes at the inner chamber walls: one pair gen-

erating a high frequency alternating current, which does not affect the animals (e.g. Kirkpatrick et al., 2006), while the 2nd noncurrent carrying pair of electrodes records the changes in the electrical field produced by the movements of the animal inside the chamber. The changes in animal behaviour can be described as changes in amplitude and frequency and allocated to typical behaviours such as locomotion, inactivity and gill ventilation. Data analysis by Fast Fourier Transformation combined with alarm algorithms based on ARIMA models, slope detectors and Double Sigma detectors provide warnimgs and alarms if the animals significantly change their behaviour or start dying due to toxicity in the water. This allows for event-related water sampling for detailled chemical profiling, hence providing spill-related analysis, which is a basis for the polluters pay principle. Like that both biological information about the toxic potential for the river biocoenosis as well as chemical diagnosis can be combined in a cost-effective and efficient win-win scenario. The MFB has been used with many different test species in various applications such as river monitoring (Gerhardt et al., 2007), waste water effluent monitoring (Gerhardt, 2019), raw water monitoring for drinking water supply (Gerhardt et al., 2003) and groundwater (Gerhardt et al., 2020).
Two Case studies show exemplarily the potential implementation of online biomonitoring in WFD monitoring and integrated water management.

(1) Bioassessment of acid mine drainage effluents (Portugal)
Water quality assessment was performed using the following biological quality components: macroinvertebrate and chironomid communities, benthic diatom community. Moreover, at the same biological assessment sites the MFB was used in situ for 48h to record the behaviour of both standard toxicity test species such as Daphnia spp. and Lemna spp. as well as local resident species such as the fish Gambusia holbrooki, the mayfly Choroterpes pictetii and the invasive macrocrustacean Atyaephyra desmarestii (e.g. Gerhardt et al., 2004, 2008). A multimetric index was developed including the following metrices: a biotic indey: BMWP, a structural index: % EPT-Taxa, a functional feeding index: % Predators, and the recorded locomotory activity as measure for fitness during five hours of in situ exposure: MFB-activity (five hours) (Gerhardt et al., 2004). The new multimetric index reflected the pollution gradient (acidity and metal pollution) better than single biotic index.

(2) Longterm river biomonitoring Rhine (Germany)
Water quality was monitored with gammarids in the streamside monitoring station of the river Rhine at the country border Germany/France/Switzerland for a period of six weeks. The gammarids were collected from a clean site of a tributary of the river Rhine, they survived the six week's monitoring period without maintenance efforts,

Figure 5: *Diatom sampling in the Una River (Bosnia and Herzegovina)*

feeding on particles in the water and an alder leaf in their test chambers. Chemical analyses were performed on regular basis. Peaks in different toxicants led to increased inactivity phases of the gammarids, hence validating the alarms given by the MFB (Gerhardt et al., 2007).
Current biomonitoring and chemical monitoring schemes consist of monitoring intervals of one to three years regarding the biological quality components and priority resp. non-priority catchment relevant chemical substances at selected sites. Hereby catchments > 10 km^2 are covered, i.e. smaller streams and brooks are not included in the WFD-monitoring at all. In these cases, investigative monitoring is proposed at sites where a significant deterioration can be expected for downstream areas.
Online biomonitoring offers serious advantages for the permanent real-time monitoring of effluents of wastewater treatment plants, industries and aquaculture effluents into rivers, at country borders and for investigative monitoring of both suspected sites and sites in small catchments. Moreover, at sites where the environmental quality guidelines for priority or non-priority substances are surpassed, online biomonitoring delivers valuable information about the toxic potential of these substances on the aquatic biocoenosis in real-time and over long periods, thus bridging between the sporadic periodic sampling dates for chemical analyses, which might only take place every one to three years. Furthermore, online biomonitoring can be installed at raw water intakes for drinking water abstraction. The asset of continuous information combined with event-driven water sampling for subsequent chemical diagnosis in alarm cases offers a dense and safe water quality control scheme of valuable sites for either biodiversity and nature conservation or for protection of drinking water, bath water and fish sites.

3.2.2. Novel genetic tools for bioassessment and monitoring of aquatic ecosystems

The organism groups including phytoplankton, phytobenthos, larger aquatic plants, benthic invertebrates and fish are known as "Biological Quality Elements" (BQEs), and used as bioindicators in the frame of the Water Framework Directive (WFD) (EEA, 2012). Monitoring and assessment methods in the Europe are still mostly based on identification using morpho-taxonomic approaches and analysis of the structure of the communities (Hering et al., 2018), except in the case of the UK, where DNA-based diatom metabarcoding approach for WFD classification of rivers is developed and applied (Kelly et al., 2018). Morpho-taxonomic identification of phytoplankton, phytobenthos and benthic invertebrates is time consuming and requires the engagement of skilled taxonomists (Haase et al., 2004). In order to simplify and speed up the assessments methods and make them cost effective, the application of metabarcoding and environmental DNA (eDNA) as the novel genetic tools for the water bioassessment has been investigated in the last decade.

The ever pressing need to reduce expense and accelerate the monitoring process has driven the scientific community to seek alternative tools which will deliver desired results without jeopardizing their quality, robustness or comparability. The expansion of use of novel genetic methods, facilitated by the advances in high-throughput sequencing technology, promised to steer the field in the direction of "biomonitoring 2.0" era (Baird and Hajibabaei, 2012; Woodward et al., 2013). Numerous academic studies employing the multispecies identification based on the high-throughput sequencing for the main bioindicators of water quality have been published: invertebrates (Hajibabaei et al., 2011; Elbrecht et Leese, 2017; Macher et al., 2016; Weigand et al., 2019; Elbrecht et al., 2017; Mächler et al., 2017), phytobenthos (Vasselon et al., 2017a; Vasselon et al., 2017b; Rimet et al., 2018; Rivera et al., 2018; Bailet et al., 2019), fish (Thomsen et al., 2012; Miya et al., 2015; Shaw et al., 2016; Hanfling et al., 2016; Civade et al., 2016), and phytoplankton (Eiler et al., 2013). A detailed overview of metabarcoding studies focusing on freshwater and marine biomonitoring is given in Pawlowski et al. (2018). However, it was recognized at fairly early stage that the vast number of different approaches was been utilized by different research groups even for the same BQEs, as was the case with traditional biomonitoring methods, thus hampering the intercomparability (Deiner et al., 2015). It became clear that standardization and harmonization of methodologies across countries was needed in order to start a dialogue about the introduction of genetics as exclusive or auxiliary tool in WFD. In that regard, a pan-European network "DNAqua-Net" (COST action CA15219) was launched in 2016 to enable utilization and improvement of molecular methods for monitoring BQEs under the requirement of WFD. Recently, Hering et al. (2018) assessed the applicability of DNA-based identification for different BQEs and water categories in WFD, based on a decade-worth of studies and taking into account all shortcomings of the approach. In Balkan countries, metabarcoding is still not applied in the term of potential use in biomonitoring. As the results of the short-term scientific mission of the DNAqua-Net COST action, a pilot project of DNA metabarcoding and morphological identification to assess diatom diversity on travertines on the river Una in Bosnia and Herzegovina has been conducted (Project No 10-33-11-340/18-11). However, instant transition to DNA-based identification in aquatic monitoring is still unattainable on a large European scale due to issues of standardization, intercalibration, library coverage and harmonization and compatibility of biotic indices. Clearly, producing barcodes for all species as well as their local populations in a matter of couple of years would be a utopian task, given the financial, infrastructural and human resources needed. This is particularly true for the less research-intensive countries such as those in the Balkans which harbor great portion of European genetic biodiversity. Therefore, a viable approach would be to prioritize filling the gaps for the important taxa on which biotic indices are calculated. As many countries in Europe still have limited sequencing and bioinformatics capacities, establishing national or (sub) regional advanced facilities for centralized analyses could be economically justifiable.

3.2.3 Application of hydromorphological elements in WFD monitoring

The basic reason for investigation into hydromorphological condition is to broaden knowledge about hydromorphological pressures and impacts caused by human activities. Hydromorphological condition is particularly important for creation of habitats in rivers. The Water Framework Directive requires that activities be undertaken for assessment of hydromorphological characteristics which are to be correlated with biological and chemical data for assessment of ecological status of waters. The determination of the ecological status of surface water bodies is based on biological elements, while hydromorphological, chemical and physico-chemical indicators are currently considered to be subsidiary elements which condition defining of type-specific biological communities in surface waters (rivers, lakes and coastal waters).

The Water Framework Directive is very limited in defining a common monitoring methodology for hydromorphological elements; due to high heterogeneity of different European regions.

Evaluation of data from the first cycle of water district management plans for the EU member states shows that 40% of the European rivers is affected by hydromorphological pressures caused by construction of dams and reservoirs, river navigation, irrigation, flood control and urban sprawl, which cause changes in natural processes (hydrological, geomorphological, and other relevant processes) and degradation of natural habitats. Hydromorphological pressures are addressed by at least one BQE in 90% of the countries for rivers, 75% of the countries for lakes and transitional waters, and in only 25% of the countries for coastal waters (Poikane et al., 2020). Fish and benthic invertebrates are the BQEs responding most strongly for rivers and lakes (Lorenz et al., 2004; Urbanič, 2014), while angiosperms and benthic invertebrates are used in coastal and transitional waters (OrlandoBonaca et al., 2012; Recio et al., 2013), and fish in transitional waters (Lepage et al., 2016). Establishment of reference hydromorphological conditions is a very complex process, since it is most frequently impossible to reverse hydromorphological modifications to a reference condition. Morphological processes frequently cause slow changes in habitats, and degradation is difficult to discover without knowledge of fluvial geomorphology. These slow changes are more easily determined by monitoring of hydromorphological than of biological elements. Therefore, it is necessary to develop a methodology for implementation of hydromorphological monitoring which will enable hydromorphological characterization and assessment of such changes, and consequently an assessment of the ecological status of waters. This would contribute to determination of measures needed to achieve at least good ecological status of water and good ecological potential of heavily modified and artificial water bodies.

3.2.4 New challenges: biodiversity – endemic species – spring ecosystem (springs of the Dinaric karst)

Today, the most endangered parts of the environment are biological and geomorphological diversity. At the end of the 20th century, the United Nations (UN), put the loss of biodiversity in the first place out of the twenty most serious environmental problems. Biodiversity has declined to the extent that we are witnessing a sixth mass extinction (Ceballos et al., 2017). Freshwaters are experiencing declines in biodiversity far greater than those in the most affected terrestrial ecosystems and if trends in human demands for water remain unaltered and species losses continue at current rates, the opportunity to conserve much of the remaining biodiversity in freshwater will vanish. The levels of endemism among freshwater species are remarkably high. Of the 29,500 freshwater dependent species so far assessed for the IUCN Red List, 27% are threatened with extinction. Among these, an estimated 62% of turtle species, 47% of gastropods, 42% of mammals, 33% of amphibians, 30% of decapod crustaceans (crabs, crayfish, and shrimps), 28% of fishes, and 20% of birds are at risk. Being aware that freshwater aquatic ecosystems are increasingly affected by the various anthropogenic stressors (e.g. hydropower, urbanization, agriculture, industry), that pollution is increasing and resulting with habitats loss, biodiversity decrease and ecosystem functions and services loss presented an Emergency Recovery Plan to reverse the rapid worldwide decline in freshwater biodiversity (WWF report; Tickner et al., 2019). This plan extends the concept of species recovery plans established in legislation such as the US Endangered Species Act 1973 and the Australian Environment Protection and Biodiversity Conservation Act 1999. The plan is structured around six priority actions (Action 1: Accelerate implementation of environmental flows; Action 2: Improve water quality to sustain aquatic life; Action 3: Protect and restore critical habitats; Action 4: Manage exploitation of freshwater species and riverine aggregates; Action 5: Prevent and control nonnative species invasions in freshwater habitats; Action 6: Safeguard and restore freshwater connectivity.

Bending the curve for **freshwater biodiversity** ultimately hinges on the extent to which effective policy and management interventions. A systemic approach to stakeholder engagement and dialogue will be needed, involving multiple stakeholders and a broad range of skills and disciplines to ensure a coherent approach to policy and planning for freshwater ecosystem management (Tickner et al., 2017). Priorities include more comprehensive, higher-resolution data on river flows and water levels, water infrastructure, water quality, and exploitation and extraction of freshwater species and materials, drawing on in situ and remote sensing technologies.

In the Balkan countries, as in Europe, springs are mainly perceived from an economic and hydrological point of view as sources of pure drinking-water. This has resulted that most springs become captured for the purpose of water supply but often also for aesthetic reasons. The effects of human-induced environmental changes are evident for many springs in the Dinaric karst. The mode of modification when tapping a spring, in general depends on the natural characteristics of a spring, especially its discharge (Stevanović, 2010), which in the Dinaric karst can significantly vary over the spatial and temporal scale. The differences in hydromorphological features of springs in the Dinaric karst have led to the existence of various transitional ecohydrological forms from heavily modified 'dammed' (forming a stone or a concrete-dammed pool) to weakly or moderately modified 'piped' springs, where water emerges from an artificial pipe (Pešić et al., 2019a). In the area of Banja Luka of Bosnia and Herzegovina and in the Skadar Lake basin in Montenegro almost 40% of springs, respectively, are heavily modified (Pešić et al., 2019a).

In the legislation of Montenegro as well as other Western Balkan countries, the springs are recognized as a water resource, but not as a habitat. Limestone Precipitating Springs (EU-Code 7220) type is the only spring type which is recognized by the Annex I of the European Union (EU) Habitat Directive, while all other spring types are out of protection. The public mainly perceives springs as water suppliers – for cattle and local communities, and very little is known about their ecological significance. Capturing springs located on land belonging to a local community or even to state ground is not prohibited. It is very common practice in the Balkans that individuals from the local communities to capture springs as part of their legacy. In recent years, the water scarcity as a result of ongoing climate change led the local communities to capture more springs. At the present, most springs in the Dinaric region, even the springs in National Parks, are not in a natural state (Pešić et al., 2019a).

In the Dinaric region the springs have been mostly investigated in the selected areas. In Bosnia, for example, the ecology of spring's assemblages have been studied by Pešić et al. (2016, 2019a), von Fumetti et al. (2017), and Savić et al. (2017, 2020). The mentioned ecological studies were related to riparian springs bordering streams and rivers, which are under strong influence of flooding events, and therefore more sensitive to the ongoing climatic changes (von Fumetti et al., 2017). In mountainous areas of the Dinaric region, springs and ecological patterns of their assemblages have been poorly investigated (Płóciennik et al., 2016). The ecology of macroinvertebrate assemblages in springs in protected alpine areas were studied in the Prokletije (Berlajolli et al., 2019). Most papers deal with faunistical and ecological research of some taxa, but not with entire communities living in spring habitats. High diversity of water mites in crenic habitats was found in the Mediterranean region of Montenegro (Pešić et al., 2019b) and Croatia (Pozojević et al., 2018, 2020). Other groups that have been ecologically studied in springs of the Dinaric region include diatoms (Dedić et al., 2015; Kamberović et al. 2019), leeches (Marinković et al., 2019), aquatic snails (Dmitrović et al., 2016; Savić et al., 2020), EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa (Previšić et al., 2007; Savić et al., 2017; Vilenica et al., 2017), dragonflies (Pešić et al., 2017; Vilenica, 2017), water bugs (Gligorović et al., 2016), water beetles (Mičetić Stanković et al., 2019), Diptera (Ivković et al., 2015) and chironomid larvae (Płóciennik et al., 2016).

Summarizing the results of the above mentioned studies on springs of the Dinaric karst, the following conclusions can be drawn aimed to help protection of these important and endangered habitats.

1. All the above studies have shown that karstic springs, despite having a small spatial extent, exhibit a high degree of individuality (von Fumetti et al., 2017) and rich communities with a large number of endemics, rare and endangered species that inhabits springs of the Dinaric karst (Savić et al., 2017; Pešić et al., 2019a).
2. The small range of spring species, and the reported decline in endemic diversity as a result of increasing human pressure (Pešić & Glöer, 2013) and the ongoing climate changes (see von Fumetti et al., 2017 for a discussion) make the spring environment particularly vulnerable. Within crenic assemblages, crenobionts, species that are strictly bound to springs and cannot be found in other types of freshwater ecosystems, are particularly sensitive (Pozojević et al., 2020). Crenobionts, most of which exhibit a particular preference for special habitats, include also the highest number of endemics and are often a priority for conservation efforts (Savić et al., 2020). Moreover, crenobionts are possibly the best indicators of the ecological status of spring habitats (Pešić et al., 2019b).
3. Response of macroinvertebrate assemblages inhabiting springs depends on hydromorphological modification of these habitats (Pešić et al., 2019a). Several studies have revealed that ecohydrological modifications of springs have a negative impact on spring assemblages of some specific taxes (Lencioni et al., 2012; Savić et al., 2017. Springs which are "moderately disturbed" such as a piped springs might harbor a rich diversity (see Pešić et al., 2019a for a discussion), even the anthropogenic activities (e.g., transformation of a river valley by preventing riparian springs from flooding) might have a positive impact on the diversity of crenobionts (Zawal et al., 2018). Therefore, springs that have undergone hydromorphological modifications to some extent, and which infact make up the largest part of springs on a local or regional scale in the Dinaric karst, are still of interest for protection (Pešić et al., 2019a).
4. Springs are not included in the standard assessment tools developed by the European Water Framework Directive (WFD). This is because the springs are considered to be too small and too different from the lower reaches of running waters (BUWAL, 1998). However, a large number of assessment methods developed for springs have emerged since the 1990s. The assessment procedures were based on the flora (Hinterlang, 1993), and the macroinvertebrates (Fischer, 1996), but also on the water chemistry (Andree et al., 1995) and ecomorphological features (Schindler, 2004), the latter assessment procedure has been adapted by Lubini et al. (2009) for evaluating springs in Switzerland. It is worth noting that all existing assessment procedures apply to the mid-European region, while for the Dinaric region appropriate assessment procedures for spring habitats have not yet been developed.
5. As shown by some studies, the quality of data used for assessing ecological status of springs, as a first step towards their protection, depends on the sampling procedure but also on the accuracy of applied biotic metrics. The results of the macroinvertebrate monthly samplings

Figure 6: Karstic springs in the Dinaric region

performed in two springs in western Kosovo revealed two important facts whose importance might be extrapolated to most of the small Western Balkan rheocrenic springs (Berlajolli et al., 2019). The first, "the multiple surveys covering more than one season provide a comprehensive picture of total biodiversity", and the second, for "appropriate characterization of the macroinvertebrate community at least winter and summer were required" (Berlajolli et al., 2019). Regarding benthic metrics, Pešić et al. (2019a) stressed that accuracy with which standard macro-invertebrate metrics characterize the ecological status of springs is almost unknown. These indices have been developed and widely used to assess the water quality of running waters (Pešić et al., 2019a), and their application in springs probably requires modification. Pešić et al. (2019a) developed a new multimetric Spring_ICMi index, which includes metrics specific for assessing the response of macroinvertebrate assemblages in springs (Pešić et al., 2019a). The newly established metric reflect both hydromorphological and physicochemical degradation, and may provide a framework for assessing the deterioration of springs at regional and possible at global level (Pešić et al., 2019a).

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