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Content

Summary

The European Union's Water Framework Directive (WFD) was initiated in 2000 and designed to provide a framework that allows for the assessment, monitoring and ultimately management of water bodies to safeguard the natural environment. In 2019, the WFD will be evaluated, and therefore it is key to assess whether emerging monitoring tools could provide cost effective means to replace or complement existing efforts to understand the status and functioning of aquatic ecosystems. This study assessed which monitoring techniques have the potential to serve surface water quality assessment under the WFD within the coming years. Techniques were evaluated on the basis of which information they effectively could provide in relation to ecosystem management and the WFD, and whether potential monitoring tools and strategies have the support of those involved in water quality monitoring and policy. Novel monitoring tools and approaches in relation to the WFD are further discussed. This study shows that several novel monitoring tools (e.g. eDNA, Effect-based tools and functional tools) were identified having the potential and support to complement existing monitoring efforts and to be implemented within either the existing WFD or a revised WFD and its inherent goals set by national authorities.

1. Introduction

The European Union's Water Framework Directive (WFD) was initiated in 2000 and designed to provide a framework that allows for the assessment, monitoring and ultimately management of water bodies to safeguard the natural environment. Monitoring efforts of water quality in Europe are thus focused on the evaluation of water bodies with the aim of improving, protecting and preventing further deterioration of Europe's water quality (EU 2000). The Water Framework Directive and its daughter directives consists of both – not necessarily linked – evaluation and protection elements; there is "good chemical status" and "good ecological status". Good chemical status is defined in terms of compliance with all the quality standards established for chemical substances at European level. The Directive also provides a mechanism for renewing these standards and establishing new ones by means of a prioritization mechanism for hazardous chemicals. This will ensure at least a minimum chemical quality, particularly in relation to very toxic substances, everywhere in the EU Community. Good ecological status is defined in Annex V of the Water Framework Directive, in terms of the quality of the biological community, the hydro-morphological characteristics and the chemical characteristics, including national and river basin-specific pollutants. As no absolute standards for biological quality can be set which apply across the EU Community, because of ecological variability, the controls are specified as allowing only a slight departure from the biological community which would be expected in conditions of minimal anthropogenic impact. This allows for the determination of the ecological health of a water body. While the WFD does not provide member states with a predetermined set of tools for water quality assessment, it ensures the establishment of adequate monitoring programs through legislative obligations to reach good chemical and ecological status for all water bodies.

Results from national and regional monitoring programs and authorities at river basin district scale are the most important way of getting an overview of Europe's water quality and the anthropogenic impacts affecting this quality. A combination of chemical and ecological monitoring allows for meeting the challenge of quickly assessing whether diffuse pollution, or substances that are authorized to be safe and hence on the market, will unexpectedly lead to ecological effects. Despite the enormous efforts, the picture that emerges regarding chemical and ecological status is still incomplete, fragmented, and with contradictory assessments of the situation. Through the one-out-all-out principle, for example, water bodies often receive 'poor' or 'bad' ecological status while in reality the degree of impairment is variable or even absent. Moreover, by focusing on those parameters that do not meet the criteria, the one-out-all-out assessment ignores improvements made for other aspects. In terms of chemical monitoring, the ability to assess pollutants is limited. Over 99 percent of pollutants as well as mixtures of pollutants cannot be measured with existing chemical monitoring methods (Brack et al. 2017). In addition, in the Netherlands, the long applied simple model of restricting emissions (more specific deleting sources of pollution) has in certain cases proven to be insufficient. These shortcomings prompted endeavors of both the scientific community and consorted water managers' initiatives to develop novel monitoring approaches and techniques.

With new techniques becoming available, the status of the environment can potentially be more efficiently evaluated: from ecotoxicogenomic changes induced by substances to the use of bioassay batteries and ecoepidemiology. In 2019, the WFD will be evaluated, and therefore it is key to assess whether emerging monitoring tools could provide cost effective means to replace or complement existing efforts to understand the status and functioning of aquatic ecosystems.

Therefore, this study aimed to assess which monitoring techniques have the potential to serve surface water quality assessment under the WFD within the coming years. To this end, techniques were evaluated on the basis of which information they effectively could provide in relation to ecosystem management and the WFD, and whether potential monitoring tools and strategies have the support of those involved in water quality monitoring and policy. This was achieved through a literature study and interviews, integrating the needs and ideas from the field, and evaluating the mutual support between science and water managers.

2. Methods

Literature search

To evaluate innovative monitoring techniques, an advanced search in the Web of Science core collection was performed on 31 October 2016 following predetermined search criteria. The following standardised search term was entered: ts=(monitoring or surveillance) and ts=(surface water or water quality) and ts=(chemical monitoring or biomonitor*) and ts=(tool* or method*) and PY=(2010-2016) and su=("Environmental Sciences & Ecology" or "Marine & Freshwater Biology") not su=medic*. When looking for specific subjects, additional topic tags were added (Table 1). In addition, relevant papers were retrieved from other sources such as interviews, symposia and EU projects.

Subject	Tag
eDNA	and ts=(eDNA or ''environmental DNA'')
Remote sensing	and ts=(remote sens*)
Bacteria	and ts=*bacter*
Fungi	and ts=(*fung* or hyphomycete*)
Invertebrate	and ts=*invertebrate*
Algae	and ts=*alg*
Fish	and ts=*fish*
Phytoplankton	and ts=*phytoplankton
Phytobenthos	and ts=(*phytobenthos* or *diatom*)
Macrophytes	and ts=macrophyte*

Table 1 Overview of additional topic tags that were used in the Web of Science search.

Comparative analysis

For our comparative analysis, a subset of the most promising monitoring techniques was selected based on their popularity and potential to improve current monitoring programs. The criteria on which the analysis is based were selected from the literature and form a range of conditions a water quality monitoring tool ideally should meet. Even though the importance of the criteria differs and partly depends on the purpose of the monitoring program in question, the criteria in our analysis are of equal weight. The evaluation of the relative performance of a variety of innovative tools for water quality monitoring was based on seven criteria extracted from the literature (Table 1): 1. sampling equipment (what are the equipment costs for sampling?), 2. sampling effort (how many man hours does the sampling require? how complex is the sampling procedure?), 3. sensitivity (ability to detect (effects of) pollutants of anthropogenic origin?), 4. analysis (how expensive and complicated is the analysis?), 5. ecological relevance (are the results representative for the state of the system? Does it include spatial and temporal scale? Can it distinguish human-induced stressors from environmental stressors?), and 6. implementation (can the tool be implemented in monitoring within 3 years and can it be embedded in the WFD? Or are modifications required?).

For analysis of the relative performance of the monitoring tools, literature was used to assign scores to the monitoring tools, based on the criteria as defined in Tables 2, 3 and 4. The strengths and weaknesses of each criterion were identified per monitoring tool from existing literature and reviews. All collected characteristics were taken in account and led to the allocation of a score that is either positive, negative or, in the case of both negative and positive scores, neutral. For groups of monitoring tools with similar purposes, such as chemical tools, scored were assigned relative to the other tools in that group. For every monitoring tool, each criterion received one of the five scores: very positive (++), positive (+), neutral (+-), negative (-) and very negative (--). An example for the allocation of scores is included below for the case of spot sampling (Table 2).

Table 2 Example of allocation of scores for spot sampling (Allan et al. 2006, Hanson et al. 2013, Bae et al 2014)

Criterion	Score	Argumentation
Equipment	++	Cheap, only tools for water sample collection are needed
Sampling effort	-	Repeated sampling required on multiple locations
Sensitivity	+-	Sensitive to chemicals, but does not cover all
Analysis	-	Extraction and gas chromatography/high performance liquid chromatography for each single compound is expensive and labour intensive
Relevance	--	Only total chemical concentrations
Implementation	++	Currently used and well established

Symposium, workshop and interviews

A symposium on monitoring tools within EU-funded projects organized by STOWA (Acronym for Foundation for Applied Water Research) was attended on 10 November 2016. At this symposium, key players involved in monitoring were identified and the primary

interest and visions related to water quality monitoring were collected. As a follow up, a workshop was organized for which in total 16 experts attended coming from different organisations in the Netherlands; the national water managers, regional water managers, data collecting organisations, governmental environmental health institutes and universities. As a follow up, interviews were held with people that are intimately involved in the monitoring of aquatic ecosystems. Key players ranged from monitoring experts working at water authorities to researchers and consultants at research institutes related to water quality. Interviews were held with different stakeholders: Laura Moria (Waternet); Leonard Osté (Deltares); Bas van der Wal (Stowa); Rob Merkelbach (Waterschap Aa en Maas); Michiel Kraak (Universiteit van Amsterdam); Hans van der Vlist (Adviescommissie Water). The workshop and interviews thematically addressed 1) Innovative monitoring tools, including: technical feasibility, accuracy and applicability of the different monitoring approaches; Integration of chemical and ecological monitoring; Use of monitoring to assess causality; 2) Data-management; and 3) Implementation of tools in relation to WFD, including costs associated with implementation; Short and long term applicability; General support for specific monitoring approaches; The role of the WFD within existing and future monitoring schemes; and Regulation of monitoring. Finally, water board managers and participants of the workshop received a follow-up questionnaire on monitoring strategies and water quality in relation to the WFD.

3. Results

Literature review – Comparative analysis of monitoring tools

Over 2000 articles were retrieved from our literature search. Over the last decades, scientific attention for monitoring tools appears to have increased exponentially (Figure 1). Figure 2 depicts the popularity of several aquatic species groups in monitoring. With 130, 114 and 109 hits respectively, macroinvertebrates, fish and algae are the groups that received the most scientific attention regarding monitoring tools. Fungi, with only eight hits, received far less attention, while bacteria on the other hand, are quite common in publications related to monitoring even though they are not included within the WFD.

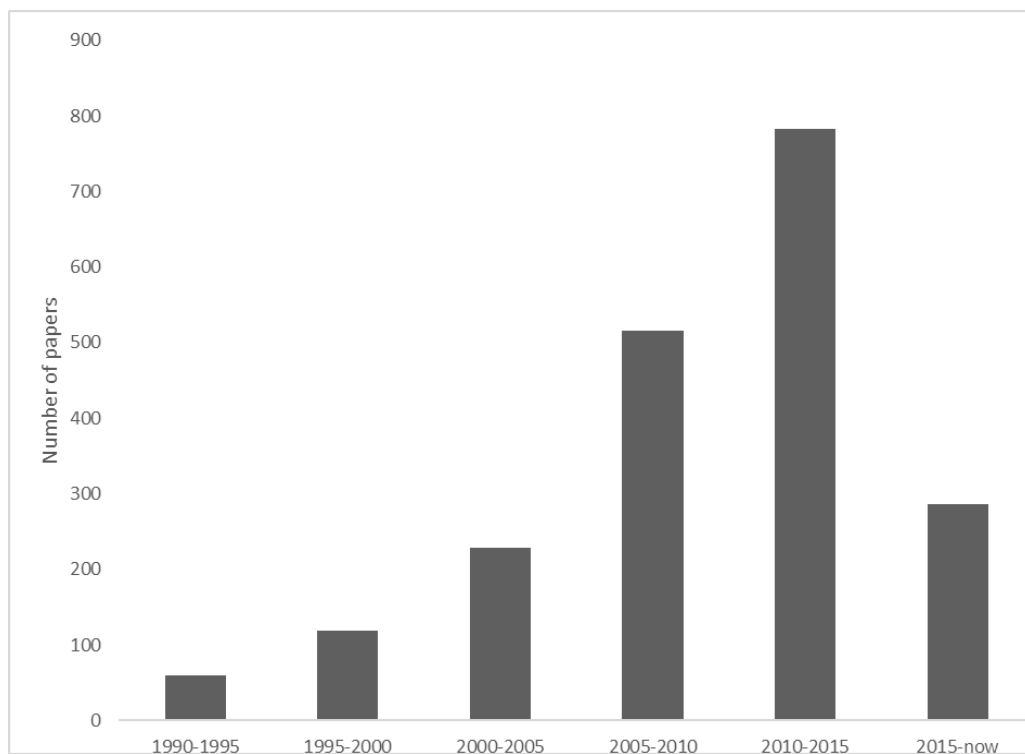***Figure 1*** *Number of publications on monitoring of aquatic water bodies retrieved from Web of Science (accessed 13-12-'16) expressed as number of publications over periods of 5 years. Note that the last bar only represents 2 years.*
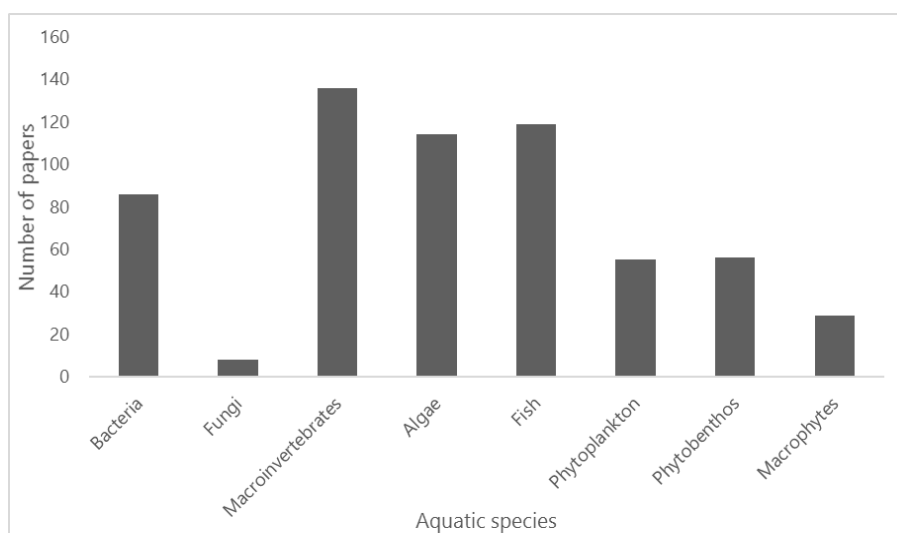

Figure 2 *Number of publications on monitoring of aquatic water bodies retrieved from Web of Science (accessed 11-1-'17) on different groups of aquatic species.*

A comparative analysis of the innovative monitoring tools based solely on the literature review is provided in Table 3. Throughout this study, it became evident that many tools were difficult to compare and difficult to rate absolutely, resulting in a comparative analysis that is somewhat arbitrary. A list of the novel tools with a brief discussion of their major advantages and disadvantages underlying their assessments is provided in Appendix 1. In this assessment, it can be noted that all tools do not necessarily score high on all assessment criteria, but overall the tools that assess the functional characteristics of a water body appear to have a better rating compared to the other monitoring tools, or groups of monitoring tools (Table 3). While accountability of a tool is also of importance, it is noted that this is virtually absent in primary literature, and hence accountability received more attention during the workshop and interviews with experts.

Table 3 *Summary of results of the comparative analysis of innovative monitoring techniques.*

	Tool	Equipment cost	Sampling effort	Sensitivity	Analysis	Ecological relevance	Implementation	References
Chemical tools	Spot sampling (WFD)	++	-	+-	-	--	++	[1]–[3]
	Passive sampling	+	++	+	-	+	++	[1], [4], [5]
	Continuous sampling	-	+	-	-	+	++	[1], [6]
Structural tools	Morphological indicators							
	Taxonomy-based (WFD)	++	-	+	-	+-	++	[7]–[9]
	Trait-based	++	-	+	+-	+	++	[7], [8]
	Interaction-based	++	--	+	+-	++	+	[7], [9]
	Environmental DNA	++	++	+	+-	+-	+-	[7], [10], [11]
	Remote sensing	-	++	+	-	+-	+-	[7], [12]
Functional tools	Ecosystem metabolism	-	++	+	+-	++	+	[8], [13]
	Litter decomposition							
	Litter bag	++	+	+	+	+	+	[8], [13]–[15]
	Cotton Strip Assay	++	++	+	+-	+	+	[7], [13]–[15]
	Decotab	++	++	+	++	+	+	[8], [13], [14], [16]
	Tea bag	++	++	+	++	+	+	[8], [13], [14]
	Wood	++	++	+	++	+	+	[8], [13], [14]
Effect-based tools	In vitro bioassay	+	+	++	+-	+-	++	[1], [17], [18]
	In vivo bioassay	+	+	+	-	+	+	[1], [17], [18]
	Biomarker	+	+	-	-	+-	+-	[1], [2], [17]
	Biosensor	-	+	+	+-	+-	--	[1], [19], [20]
	Biological Early Warning System	-	+	+	+	+	+	[3]

Table 4 *Brief description of the assessment criteria used in the comparative analysis of innovative monitoring tools. See method section for further details.*

Assessment criteria	Definition
Equipment cost	Cost of the equipment needed for sampling
Sampling effort	Labour intensity and complexity of the sampling method
Sensitivity	Ability to detect (effects of) pollutants of anthropogenic origin
Analysis	Cost and labour intensity of the analysis
Ecological relevance	Ability to provide insights which are relevant under natural conditions and representative for the state of the aquatic system
Implementation	Possibility of application in the Water Framework Directive within three years

Interviews & Workshop

Generally, concerns raised by the experts were that current measurements of priority substances do not take into account the enormous amount of emerging substances and effects of pollutant mixtures, transformation products or interactions between chemicals. What is considered necessary is a set of complementing tools that provides information on different aspects of water quality. Hence, tools desired should often full-fill many different aspects such as continuous monitoring or long-term and screening-wide endpoints in both the chemical concentrations and ecological responses. Being aware of the existence of certain techniques was often key for, for instance, water managers to select them, in that respect passive samplers – although not meeting all assessment criteria in the literature review – were often seen as integrative and useful measures.

Overall, a tool was considered potentially useful when it fits the requirements for reporting under the WFD. Given the financial burden of these requirements, this was generally considered more important than the potential use of a tool to better understand and manage the aquatic ecosystem under focus. For instance, the classification of ecological status within the WFD requires information on biological, hydro-morphological and physicochemical elements. Current ecological status classification based on taxonomy is considered laborious and expensive, and not providing a representative picture of water basin health. Hence, the use of for instance environmental DNA (eDNA) is considered to be valuable to expedite the ecological classification of aquatic water bodies. eDNA is welcomed by water managers, because it substantially improves the ability to detect species otherwise missed with traditional sampling methods. Ever advancing Next Generation Sequencing (NGS) techniques make analysis possible for limited cost and effort and sampling can be combined with the collection of samples for chemical monitoring, reducing costs even further. This technique therefore seems readily adopted in monitoring programs within three years to provide additional information on vertebrate species and its development is strongly encouraged by water managers. However, this approach to date does not allow for an accurate quantification of entire food webs, and thus does not necessarily increase an understanding of the aquatic system, nor does it enable water managers to take appropriate measures for restoration, as required under the WFD.

Incorporation of effect-based tools or more functional indicators such as functional trait composition of the aquatic communities or processes like production and decomposition are considered to be more valuable by the scientific experts. However, for process-based indicators to be adopted within WFD monitoring schemes, the WFD criteria for ecological status classification must be adjusted and the use of these indicators must be encouraged by water authorities. Whereas novel effect-based tools or structural tools are easily accepted by water managers, implementation of process-based indicators seems to require more top-down reinforcement. Either way, process-based indicators can act as a valuable complement in water quality monitoring and with some adjustment of WFD policies these tools can be adopted in WFD monitoring in three years

With the use of molecular tools and other big data tools such as remote sensing and continuous monitoring, the volume of generated data increases exponentially. This leads to an increase in data, complexity of data, and need for software and bioinformatics. Nonetheless, it is considered essential to guarantee database quality through correct data management, and to keep data consistent to allow for long term monitoring of changes in water quality and evaluating management and policy. Although the field of data management is rapidly progressing, current efforts to standardize and centralize data appear insufficient, rendering existing databases useless for trend-analyses.

Incorporation of additional monitoring measures imposes a financial burden, and hence the general notion is that the implementation of novel tools should rather replace existing methods than complement them. It is considered important by all interviewed experts that a transition or extension in monitoring schemes should be financially supported by, for instance, national governments. Moreover, a disparity seems to exist between the monitoring tools that are developed in both the applied science (waterboards, STOWA) and fundamental science (research institutes and universities) arena. Applied sciences mainly develop tools that assist, improve and/or ease-up the requirements for reporting under the WFD (bottom-up fueled). In contrast, fundamental sciences mainly develop methods that are designed to improve an understanding of the actual water quality (science fueled). This suggests that there is a need for an improved communication between water policy and management, and better integration or rethinking of policy requirements and ambitions to safeguard the environment.

Response to questionnaire
(sent to workshop participants; water boards, national water managers)

Water board managers and participants of the workshop received a follow-up questionnaire on monitoring strategies and water quality in relation to the WFD. A total of 7 responded, and their responses can be summarized as following *(questions in italics; responses in normal font)*:

1. *If you were not restricted by the legal framework of the WFD but only having a limited budget, how would you organize monitoring and what is the main motivation for that monitoring?* To better understand the aquatic system and entangle how generic policies and specific measures influence the water quality (so how much we gain from our efforts that cost a lot of money), monitoring strategies preferably provide a complete picture of the situation

and development of an area / water, e.g. a systems analysis. Most people gave their idea on the monitoring scheme which was often seen as a cycle of three to five years, in which each year emphasis was put on a different focus. One year of generic overall screening of many different chemical compounds, followed by two years of monitoring only at strategically places (those that were seen to be vulnerable or those with contaminant concentrations exceeding the quality standard) using spot sampling, passive sampling and effect-based samplings (bioassays). The chemical monitoring is then aimed to find emission sources. Innovative monitoring is considered valuable if it would provide an ecosystem wide understanding of (multiple) process(es).

2. *If you are restricted to the legal framework of the WFD, but can have more choices which monitoring activity would you then skip or use differently?* In general, the responding water managers would like to spend less money to chemical monitoring (and advocate that emissions could also be regulated by Waste Water Treatment facilities) and predominantly focus on effect-based monitoring. Additionally, this question was mostly addressed by people giving specific examples – which suggests that the generic answer on this question is that people would like to spend their money on site-specific monitoring. Examples are: Part of our regional budget is required for monitoring that is irrelevant for the specific area. For instance, (chemical) spot sampling of hydrophobic chemicals which are year after year below detection limits (we prefer passive sampling or sediment sampling or any alternative that gives valuable info), or (ecological) fish monitoring in water bodies where no measures are implemented. Some water managers / experts like to modify the monitoring by using adaptive approaches. In other words, as soon as the system is understood, and parameters are in compliance with the water quality targets, these parameters could receive less attention in monitoring efforts (for instance assessing only in a three to five year cycle). Available resources could in these cases be used to perform targeted monitoring fueling the information needs of that specific area.

3. *Which of the above-mentioned changes is achievable to be changed in the WFD within three or ten years?* Within 3 years, passive sampling, drones, bioassays, eDNA all will be likely enrolled within the WFD, as well as more emphasis on effect-based measurements. Continuous measurements (physical / chemical) can provide much more insight into the water system. At the same time, according to some experts the WFD (in 10 years) would benefit from an ecosystem approach that does not consist of quality elements, but on a more system-oriented way of managing areas. For instance, areas with anthropogenic impacts (by industry and agricultural sector) require fundamentally different practices, because achieving a high water quality in such areas would require fundamental choices regarding the (economic) activities.

4. *From the perspective of citizens, which (potential) monitoring strategies do you consider important?* Often people are mostly interested in clean water respecting no litter or any other forms of smelly water or excessive duckweed and algae, in which water bodies allow for swimming and fishing is considered of prime importance. In this case, it is considered sufficient to have visual inspection. Underlying nitrate and phosphate levels in the

water and hereby innovative techniques allowing citizens to measure is considered helpful (in the communication and participation), i.e. developing App's for smartphones. In addition, remote sensing and fast data generation is also considered important.

5. *Respondents were also requested to judge whether novel tools as depicted in Table 3 could potentially serve a better understanding of aquatic ecosystems and inherent management, as well as their suitability considering the constraints of the WFD. Respondents were requested to rate specific tools in aspiration of obtaining an average rating of tools with respect to a tools potential to increase an understanding of aquatic ecosystems and inherent management, as well as their suitability considering the constraints of the WFD.* In many cases, respondents refrained from giving a rating, and rather assessed specific tools of sets of tools in wording. This limited our ability to present overall ratings that could for instance serve to complement Table 3. Despite this, it could be noted that respondents used their positive views on the criteria implementation predominantly to support the notion that a tool or maybe preferably a set of tools is considered potentially useful when it fits the requirements for reporting under the WFD – e.g. eDNA.

4. Discussion

Short term - Promising monitoring tools

Even though quite a few innovative monitoring tools have emerged after the introduction of the Water Framework Directive, most of these methods are not yet implemented in present water quality assessment. For a tool to be suitable for water quality monitoring, they should be cost-effective, highly standardised, should and comprise both temporal and spatial scales. In addition, chemical monitoring tools should be able to quickly detect a broad range of (emerging) substances, with different properties. Ecological monitoring tools should be able to integrate different organisational levels. All monitoring tools fail to meet all these criteria or require additional research before they can be implemented in the field. Moreover, throughout this study, the aforementioned criteria were often not determinative in the support or judgement of water board members and experts on whether specific tools of approaches were useful for implementation. Nonetheless, considering both the results of the literature review and the opinions expressed by a large number of experts and stakeholders throughout this study, a number of emerging monitoring tools seem to have the potential to serve water management, especially within the current boundaries of the WFD.

For WFD chemical monitoring, member states require information on concentrations of 45 priority substances and a selection of national and river basin specific chemicals. However, this does not take into account the enormous amount of emerging substances which are not listed and thus are not measured and evaluated within the context of the WFD. In addition, while many water boards would consider measuring emerging compounds, the information required for implementation of such monitoring efforts is often lacking. Furthermore, existing chemical assessments do not include pollutant mixtures, transformation products or interactions between chemicals, and have high detection limits. What is needed is

a set of complementing tools that provides information on different aspects of water quality. A promising tool in this context is passive sampling. Through providing a time-integrated measure of truly dissolved substances, even at minor concentrations, passive sampling is an effective means of monitoring which fits perfectly in WFD chemical monitoring, in particular by complementing the classification of chemical status. For non-polar substances (and increasingly for polar substances), passive samplers are adequately developed for monitoring purposes and even though total monitoring costs are higher, the price/quality ratio is positive (Smedes et al. 2010). Since this tool is sufficiently developed for monitoring purposes, passive sampling has the ability to offer valuable information in WFD chemical monitoring within a period of three years.

Effect-based tools currently seem to have the potential and required support to complement chemical monitoring. This would establish a link between pollutants and their effects on aquatic organisms by taking into account additional substances and mixture effects that are not captured by existing chemical monitoring schemes. Effect-based tools can be used in a tier-based way, in which the first tier, for instance *in situ* cages using either *in vivo* or a Biological Early Warning System, in which an organism is used as an alarming indicator for contamination, serves as an early warning for the presence of a wide range of pollutants. Systems like this are already used to assess drinking water quality (Bae & Park 2014) and can easily be adopted in WFD surface water monitoring within three years. If, for instance, bioassays, do not detect pollutants, there is, depending on their sensitivity, no need to proceed to extensive investigative monitoring. Biomarkers can be worthwhile in a second-tier assessment to confirm responses to stress. Lastly, the third tier is used to identify the cause of observed toxicity through extra analysis in the form of Effect Directed Analysis (EDA) or Toxicity Identification Evaluation (TIE). To diagnose the cause of observed toxicity, both EDA and TIE can be useful in WFD water quality assessment, either through identification of emerging compounds or through assessment of regulated substances. An example of this kind of tier-based approach is the ecological key factor Toxicity ('Ecologische Sleutel Factor', ESF, Stowa 2016) developed by STOWA. This method is an iterative, tiered framework based on bioassays and chemical assessments and is expected to be available for application in water quality monitoring by the end of 2017. This type of tiered approach is currently stimulated by STOWA and STW (applied science funding scheme) projects (Van der Wal), and the SOLUTIONS project (Posthuma). ESF receives support from water boards, and provides a clear and comprehensible tiered-based approach for assessing water bodies. The trivialization it presents also seems to carry a pitfall as there seems to be an increasing notion that in certain cases it might too much rely on deceptive oversimplification. For instance, the first three ecological key factors aim to gain insight in the restoration of submerged aquatic plants by looking at light and nutrients in the water and sediment. However, they do not account for interactions between aquatic plants and other species. Thus, although the ESF approach is promising, it requires a better integration of ecological complexity to provide reliable assessments of water bodies.

Current ecological status classification based on taxonomy does not provide water managers a solid understanding of ecosystem health to take required measures to manage and restore ecosystems. Incorporating traits (e.g. body size, reproduction, dispersal) and interactions within ecological monitoring has the potential to improve ecological spot

sampling by offering more complete information on the community as a whole. The functional trait approach is a spatially robust means of measuring change in community structure and can be implemented in current monitoring programs with little effort and within the period of three years, because it only requires the allocation of species to functional groups.

A rapidly developing tool that seems welcomed is the use of environmental DNA (eDNA), although its current potential to assess the structural composition of aquatic communities is still very limited (see Table 3). Despite its limitations, experts and water boards consulted in this study do consider this technique particularly useful for the presence/absence screening of indicator species, therefore also fitting the current WFD and inherent requirements to report under the WFD. Full community characterization using eDNA, however, is still in its infancy, but could be useful on the long run (~10y) as it is rapidly developing. Additionally, eDNA can be used to characterize microbial communities, which play a key role in nutrient cycling and support numerous ecosystem processes, but are currently overlooked in current water monitoring programs.

Long term - Promising monitoring tools

Throughout this study, novel tools were mainly considered worth pursuing if they would fit the reporting requirements under the WFD. However, ecological monitoring would have the potential to provide a more representative understanding of an aquatic ecosystem when existing monitoring schemes would be complemented with indicators based on ecosystem processes. While functional processes are essential for ecosystem functioning, existing classification of ecological status is primarily based on structural measures and indicator species. Monitoring species offers valuable information on the ecological state of a specific water body, process-based indicators such as decomposition and ecosystem metabolism can also offer a highly ecologically relevant means of monitoring, enabling spatial as well as temporal comparison. For process-based indicators to be adopted within WFD monitoring schemes, the WFD criteria for ecological status classification must be adjusted and the use of these indicators must be encouraged by water authorities.

In line with the proposed tiered approaches in effect-based chemical monitoring, the ecological focus of tier-1 could be a tool that describes the functioning of a specific water body. This approach currently receives little attention, especially in applied science institutes, but is well advocated in the scientific literature. This is mainly due to the fact that functional indicators, in contrast to effect-based tools, better capture ecologically relevant interactions and processes. However, as in most ecological monitoring tools, it remains a challenge to derive accountability criteria, threshold values or reference conditions or process rates. These criteria increasingly rely on quantitative approaches, yet remain arbitrary, and the question remains whether deriving accountability criteria ultimately serve the pursuit of acquiring a healthy status and functioning of water bodies.

Within the classification of ecological status of water bodies, the incorporation of spatial and temporal complexity also already proved to be particularly useful. Real-time continuous monitoring platforms can be deployed to track and predict harmful algal blooms as

demonstrated by the Dutch water authority Brabantse Delta. Combined with telemetry, these stations can act as an early warning system, providing the opportunity to rapidly come up with appropriate measures. Furthermore, real-time platforms provide important information on fluctuations over time of physicochemical parameters, such as pH, temperature and conductivity, which influence biological communities. Remote sensing using drones or satellites offer a similar understanding and predictive potential of the spatial complexity of aquatic water bodies. Likewise, novel molecular techniques can generate valuable information on the effects of environmental stressors on aquatic ecosystems. This wide variety of 'OMICS'-techniques, ranging from protein level (i.e. proteomics) to RNA level (transcriptomics) can be developed to predict effects or elucidate a pollutants mode of action. However, whereas novel structural tools are easily accepted by waterboards due to reporting requirements under the WFD, there seems to be a lack of support to implement spatio-temporal complexity or process/OMICs-based indicators in monitoring schemes. This seems mainly due to uncertainties on how data obtained from these approaches reflect the ecological status of water bodies. This suggests that a rethinking of the WFD and/or translation of the WFD by national authorities is required to increase support of local authorities responsible for quality assessments.

WFD & Water management in the Netherlands

Implementation of the WFD

Current efforts to monitor the Dutch and European aquatic environment appear largely fueled by the European Union's Water Framework Directive (WFD) initiated in 2000. Throughout this study, it became clear that a tool was considered potentially useful when it fits the requirements for reporting under the WFD. Given the financial burden of these requirements, this was generally considered more important than the potential use of a tool to better understand and manage the aquatic ecosystem under focus.

Although several tools have the potential to be enrolled within the WFD (whether within three years or on the long run), the WFD and its implementation currently uses an ecosystem-wide approach that does not differentiate local quality elements. This seems to be in line with opinions of experts in this area: always develop more simplified tests or SMARTER monitoring, yet keep in mind that this is not the solution to our water quality challenge. The challenge at hand is considered to seek innovation and awareness. For instance, industrial and agricultural practices are fundamentally different with unique effects on their immediate environment. This is a relevant aspect of water management in the Netherlands, which contains a large volume of water and intensive agricultural practices that can be specific to a confined area. It seems that integration of these components received insufficient attention in Dutch water quality legislation, as it currently does not include an evaluation focused on a specific area or consideration of land use practices. Important here is that the WFD offers a framework and guidance for assessing water quality. Hence, there is considerable freedom within water quality assessments, yet its implementation by national

governments and how things are perceived by local water boards ultimately seem to impose substantial constraints.

In the Netherlands, Water Management agreements (in Dutch 'Bestuursakkoord Water', abbreviation BAW), signed in 2011, run until 2020. The BAW is one of the policy tools that complements and the planning cycle required by the WFD (among National Water Plan). An administrative agreement is an instrument that suits the situation where water management is decentralized. The commission Peijs evaluated the BAW in 2013 and concluded that "the BAW as a tool has proven to work well for the common Dutch challenge to achieve a good water quality". This finding represents a promising starting point for the new issues in the Dutch water management that require a common approach. The Water Advisory Committee issued an opinion on July 14, 2016 (Van der Vlist, AcW.-2016/148398) to the Minister of Infrastructure and the Environment on the updating of the Administrative Agreement on Water (BAW). The focus of the advice is on the accountability of the agreements that still can be increased by the reformulating objectives so they are stricter and agree on a timetable in which the results to be achieved. The ministry ((JENMJBSK-2016/1 16064)), dated 10 June 2016, positively responded to this, stating that regional solutions are required for groundwater and water protection, for example, in relation to the local land use from agriculture, and in more stricter management around medicine residues. In addition, the advice made by the Van der Vlist-committee to invest pinpoint responsibilities, and more strictly formulates objectives which particularly should be in control by regional water managers, was also endorsed by the ministry.

Throughout this study, a number of experts expressed their concerns regarding whether continuation of the current WFD and goals set by the national authorities ultimately would result in the aspired good ecological quality of water bodies in the Netherlands in the next decade – a concern that was also expressed in recent report by the Water Committee (2016). The current situation does not consider area specific social and economic benefits of land use practices and inherent utilization of water bodies, hampering proper assessments in the Netherlands where virtually all territory is devoted to agricultural and industrial practices. Expecting that all water bodies will be able to reach the conditions and communities of a pristine water body is thus likely too ambitious according to those experts. Not accounting for a functional differentiation is therefore generally expected to result in many Dutch water bodies failing to meet the criteria on the long term. Some of the experts propose to differentiate between water bodies in the Netherlands with full reconsiderations of the watershed-specific setting, including the desired function of the water body, thereby mimicking current approaches for soil in the Netherlands. However, it should be noted that unlike water quality, soil policy is not subject to European law and there are much more options to develop national policies.

In addition, national authorities are, with respect to state of the art knowledge and ongoing developments within the area of monitoring and management of aquatic ecosystems, relying on research institutes, applied science consortia and universities. Knowledge is therefore considered to be scattered, ambiguous, inconsistent, and partially communicated, suggesting that future efforts to implement the WFD by local authorities requires inputs from a consortium that develops knowledge-sharing and project portfolio.

Various chemical policy frameworks, such as the EC Regulation (1109/2007) on plant protection products, the EC Regulation (528/2012) on biocides and the REACH regulation (EC 1907/2006) on industrial chemicals aim to the protect human health and the environment through an early identification of the intrinsic properties of chemical substances and their potential risks (pre-market registration and/or authorization). These substances legislations are examples of *prospective* risk assessments.

In current nature policies and compartment-oriented policies (e.g. WFD, soil and sediment policy) the attention for pollution exists, but is addressed independently of the chemical regulations. For example, the WFD asks the water quality to fit to the toxic-chemical quality standards (= concentration limits) for a set of compounds, but also to the concept of Good Ecological Status. Whatever the cause of ecological impacts (toxic chemicals or other causes), water managers should define Programs of Measures to reach the desired status (Posthuma et al 2008). The evaluation of those aspects by performing monitoring in the context of the WFD is filled in by both chemical and ecological monitoring as earlier discussed, and can be seen as *retrospective* risk assessments. There are many crucial differences that can be identified between prospective risk assessment within chemicals legislation and retrospective chemical and ecological monitoring. These differences are important to identify, because they may explain those cases where risk assessment may fail to curtail the adverse effects of chemicals on biodiversity and associated ecosystem services. In the case of plant protection products, these differences foremost include: 1) ecological recovery may be considered within the approval of active substances versus the "no effect" quality criteria in the WFD. This may result in higher regulatory acceptable concentrations compared to the predicted no effect concentrations; 2) single substance authorization versus the ecological water quality monitoring that considers by definition joint impacts of chemical mixtures and natural stressors; 3) deviating spatial resolutions, and 4) deviating ecological endpoints, i.e. model test species are typically well-studied organisms that have "easy-to-culture or maintain" characteristics, yet not necessarily are the most vulnerable species as identified based on mode-of-action (a detailed description of differences PPP approval and WFD is described in Brock et al 2009 and the RIVM report 601714026/2014 written by Smit and Kalf 2014. A brief summary between prospective risk assessment and retrospective chemical and ecological monitoring is provided in Appendix 2). Potential solution to these issues could be found in more harmonization of prospective and retrospective risk assessments. For plant protection products this could be achieved, for instance by:

- Harmonization of the ecotoxicological dataset used for risk assessment;
- The post-registration monitoring enforcement to follow ecological recovery within the agricultural drainage ditch;
- Exposure calculations based on multiple paths, so extent the direct drift emission route with direct run off, and long term leaching prospects;
- Tune the evaluation of emissions within small agricultural ditches with the larger waterbodies in which multiple agricultural and non-agricultural ditch systems collect their water;

- Use monitoring data within the (re)-evaluation of the authorized chemicals is nowadays done but on a small scale and is not fully implemented within the regulations yet;
- Consider mixtures within the risk assessment of the single compounds;
- Retrospective assessments or monitoring of eDNA focusing on organisms used for prospective risk assessments (e.g. algae, daphnids and fish).

These actions are all within the idea that agricultural actions should have negligible impact on the water quality. Obviously, alternative options exist, for instance working towards a clean water by separating area-functions and discarding the idea that ecosystems harbor multiple functions. This option was suggested by a number of experts in our interviews. Further discussion on this topic is, however, beyond the scope of this study.

5. Conclusions

The European Union's Water Framework Directive (WFD) was designed to provide a framework to safeguard the natural environment. In 2019, the WFD will be evaluated, and therefore it is key to assess whether emerging monitoring tools could provide cost effective means to replace or complement existing efforts to understand the status and functioning of aquatic ecosystems and whether potential monitoring tools and strategies have the support of those involved in water quality monitoring and policy. Several novel monitoring tools could be identified that have the potential and support to complement existing monitoring efforts, by either increasing our understanding of the structural composition of aquatic communities (e.g. eDNA) or provide a more targeted insight into the pollutants and mechanisms underpinning the effects observed in the field (e.g. Effect-based monitoring). These tools have the potential to be implemented within the existing framework of the WFD. However, it also becomes evident that, while the WFD was designed to assist in aquatic ecosystem management, its reporting requirements evolved into a legislative and financial burden that does not necessarily provide the understanding required to manage and mitigate threats to the natural aquatic environment. It is expected that continuation of the current framework will unlikely translate into a good ecological quality of the aquatic environment in the Netherlands in the next decade. Long term assessment and management of water bodies would therefore benefit from a rethinking of the WFD and its inherent goals set by national authorities. Within this, it remains important to realize that the ultimate goal is to provide a framework to safeguard the natural environment, and that no monitoring tool or approach, nor arbitrary derivatives of ecological or chemical status in itself is likely to fulfill these requirements, but may at best assist in reaching this aim. A more flexible, tiered approach that allows for the identification of causality, as well as harmonization of the authorization of chemicals with the chemical and ecological monitoring therefore offer potential solutions.

6. Acknowledgements

7. References

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Appendix I. Description of evaluated chemical and ecological monitoring tools

This appendix lists the various monitoring tools derived from primary literature used for the comparative analyses and subsequent discussions with water boards and experts during interviews and workshops. Tools are sorted in the same way as in the main text.

Chemical monitoring

Chemical status of a water body is based on comparison of chemical concentrations against Environmental Quality Standards (EQS). Under the WFD member states are obligated to measure 45 priority substances and a selection of river basin specific chemicals with a frequency of once a month and once every three months, respectively (EU 2013). Water samples for chemical analysis are usually collected through grab sampling. This method provides a snapshot of pollutant presence and cannot give long-term information on pollution. Even more, where concentrations fluctuate over time, spot water sampling do not capture pollutant peaks and may not provide trustworthy information.

The WFD offers Member States the option to derive their own EQS for substances in biota or sediment (EU 2010). In sediment and biota chemical monitoring concentrations of priority substances are obtained through analysis of sediment or bio indicator species tissues, mostly of fish, crustaceans and molluscs. Aquatic organisms and sediment to a certain extend integrate pollution of a water body in space and time and are influenced by environmental and biological factors, which results in an estimate of bioavailable concentration. Even though this method requires a lower sampling frequency than chemical monitoring and provides more ecologically relevant information, biota and sediment are rarely used in the Netherlands as a matrix for WFD chemical analyses. In the end, the choice of matrix depends on the physicochemical characteristics of the substances being monitored and the water body in question (EU 2010). When combined with traditional water matrix sampling, biota- and sediment monitoring can offer a comprehensive image of the chemical status of a water body.

Even though chemical monitoring has its limitations, information on pollutant concentrations provide evidence on analysed pollutants and trends extracted from long time series of data can assess the level of risk in a water body, enabling effective regulation of emissions of specific substances (Wernersson et al. 2014).

1.1 Passive sampling

Complementary for or even replacing biota and sediment monitoring is passive sampling, an in-situ method based on pollutant accumulation through diffusion. Over a period of hours to weeks pollutants accumulate into the sampler and are subsequently eluted and measured in the lab, resulting in time-weighted average (TWA), which forms an indicator for exposure to aquatic organisms (Lohmann et al. 2012). The time-integrated measure of truly dissolved substances obtained by passive sampling includes chemical activity and provides relatively longer-term insights, potentially on mixture toxicity.

Passive sampling enables long-term comparisons with lower frequency sampling than conventional chemical analysis, thus reducing costs and labour intensity. Another benefit associated with this sampling method is the detection of pollutants present at very low

concentrations. Although peak concentrations are included, they are averaged and therefore the exact size and timing of the peak cannot be determined (Smedes et al. 2010). Furthermore, translation from sampler uptake to pollutant concentrations can be quite complex and may require pre-treatment and additional analysis.

Unfortunately, current passive samplers for water quality monitoring are mainly suited for hydrophobic substances. Most of the emerging compounds are polar, for which many uncertainties exist in the translation of lab calibration to the field, making accurate estimation of hydrophilic pollutants difficult (Smedes et al. 2010). SinceOverall, the combination of time-integrated measurements with well-defined sampling matrix properties and low limits of quantification makes passive sampling a tool that fits perfectly in present water quality assessment.

1.2 Continuous sampling

Since water quality parameters fluctuate over time, traditional spot sampling comes with the risk of acquiring an unreliable picture of water quality. Continuous monitoring, i.e. frequently repeated measurements at discrete time intervals, results in a nearly complete record of changes in water quality, capturing diurnal, seasonal and event-driven trends. The frequently repeated measures are carried out by unmanned platforms with several sensors attached which measure mostly physicochemical parameters. Temperature, specific conductance, pH, dissolved oxygen and turbidity are parameters most commonly measured, but continuous monitoring can also be applied to measure oxidation-reduction potential, water depth, ammonia, nitrate, chloride and fluorescence (e.g. Vorenhout et al., 2011). At present, there are no available sensors (yet) for measurement of WFD priority- and river basin specific substances.

Even though the monitoring stations are unmanned, they are not entirely without effort. The platforms and its equipment require maintenance, sensors must be calibrated, a periodic verification of sensor calibration and a labouratory needs to be set up at a secured site (Wagner et al. 2006). Even more, monitoring disruptions such as equipment malfunction, bio-fouling, debris and vandalism require additional visits. Continuous monitoring provides vast amounts of data, which require complex and consistent procedures to separate valuable information on water quality from useless data.

Lately, a couple of projects (e.g. NETLAKE and GLEON) were set up with the aim to create a network of in-situ, real-time monitoring platforms by e.g. standardisation in data processing, collection and quality control. In combination with emerging sensor technologies, these projects increase the potential of continuous monitoring for WFD substances.

Ecological monitoring

In its broadest definition ecological monitoring is the assessment of biological responses to understand and track changes in the environment with the aim of protecting ecosystem services and goods. The major benefit of ecological monitoring is the high ecological relevance: it integrates effects of pollutant mixtures, interactions between

chemicals and bioavailability and offers comprehensive information on ecosystem structure and functioning.

Water Framework Directive ecological monitoring considers biological quality elements (BQEs) and hydro-morphological, chemical and physicochemical elements that support the BQEs. The BQEs form the basis of the ecological status assessment and consist of abundance and diversity measures of fish fauna, macroalgae, phytobenthos, phytoplankton and benthic invertebrates (EU 2005). Assessment of ecological status through BQE measurements follows the 'one-out-all-out' principle: the worst result for one of the quality elements determines the overall ecological status (Arle et al. 2016). Through combining information on biological, hydro-morphological and physicochemical elements and comparing these values to reference conditions, a water body receives one of the five ecological status classes: very good, good, moderate, poor or bad. When the status is defined as moderate, poor or bad, appropriate measures have to be planned and implemented in order to reach the WFD objective of a 'good ecological status'.

A drawback associated with ecological monitoring is that effects of human-induced stressors go unnoticed until a community is significantly affected, which can take a long time. This can be resolved by using methods with an early warning function, such as the Biological Early Warning System (BEWS). An additional disadvantage is combined effect of stressors, which make it challenging to determine the cause of an observed effect. Due to the influence of naturally occurring factors, results must be compared to unaffected control conditions. These reference sites act as replicates to assess natural variability and enable assessment of human-induces stressors. However, reference sites that are not affected by anthropogenic pressures are scarce, so usually reference conditions are based on sites with a minimum level of human impact. However, with this approach there is no guarantee that reference sites can be comparable across member states.

Current ecological monitoring relies mostly on taxonomy and less on traits or ecosystem functioning (Birk et al. 2012). Yet, a considerable amount of scientific literature on the development of new ecological monitoring tools has been published. The strengths and weaknesses of a selection of these innovative monitoring approaches for ecological status assessment is discussed below.

2.1 Structural indicators

2.1.1 Morphological indicators

The vast majority of indicators used in freshwater ecological monitoring programs are based on community structure. Since communities integrate effects of stressors over time and space, structural metrics provide substantial information which cannot be achieved by physicochemical data alone and are therefore valuable to water quality assessment.

Most of the structural metrics include macroinvertebrates, since they are ubiquitous, sensitive to a range of stressors, underpin many ecosystem processes and their taxonomy is well described. Because of these benefits, many structural metrics have been developed for macroinvertebrate communities. Other taxa included in structure-based monitoring are macroalgae, phytobenthos, phytoplankton and fish. Ever advancing molecular techniques

open the possibility for adopting reliable fungal and bacterial community metrics in WFD ecological monitoring in the near future.

The process of collecting, separating and identifying individuals can greatly influence results and subsequent management considerations (Carter & Resh 2001). For macroinvertebrates alone numerous sampling methods have been developed. In most cases each country has its own sampling methodology and assessment system. However, in order to enable comparison between biogeographical regions, a universal set of macroinvertebrate measures needs to be adopted. Furthermore, the ideal method needs to be as simple as possible providing sufficient accuracy and precision at minimum costs. In practice, most existing methods for macroinvertebrate sampling have evolved from the same ancestors (Saprobic index and Trent index) and it has been shown that the different approaches can be intercalibrated relatively easy (Friberg et al. 2006) hopefully resulting in a uniform measure of structural indicators in the near future.

The majority of the approaches apply net sampling, a conventional means of sampling based on visual detection and counting of individuals and therefore an inaccurate and labour intensive method. The use of activity traps on the other hand is a form of passive sampling, reducing labour intensity and costs and preventing operator bias associated with net sampling (Verdonschot 2010). Traps are deployed in the water for a fixed period of time and result in a measure of activity-density of mobile macroinvertebrates that includes diel differences in activity. To what extent mobility and activity of individuals and population density influence the capture rate is unknown, just as the effect of mortality and strong interactions between captive individuals, especially with increasing deployment time.

2.1.1.1 Taxonomy-based indicators

There has been a tradition in water quality assessment to monitor structural indicators based on the identification of species using morphological characters. Biological quality elements measured under the Water Framework Directive are no exception. These measures of diversity are useful for tracking and comparing ecosystem state and quantifying drivers of change, but they cannot link cause to effect or disentangle multiple stressor effects. The identification of organisms to species level requires a high degree of taxonomic expertise and with increasing detail, costs add up. Besides identification difficulties, biogeographical uniqueness constraints comparison among biogeographical regions.

As with the wide variety of sampling methods, numerous regional indicators for water quality based on taxonomy have been developed. In the end, a uniform measure of change in biodiversity using the strengths of existing taxonomy-based indicators would offer valuable information on ecosystem structure and in combination with a standardised sampling method, can be an important tool in water quality assessment.

2.1.1.2 Trait-based indicators

Recently, there has been a shift towards trait-based indicators. Species drive processes in various ways, depending on their functional traits (e.g. body size, locomotion etc.). Theoretically, trait-based metrics offer a link between structure and function. Even more,

species traits relate directly to environmental variables and understanding how abiotic factors influence communities brings us closer to linking cause and effect (Jackson et al. 2016).

Rooted in ecological and evolutionary concepts, trait-based indicators offer a mechanistic alternative to taxonomy-based indicators and provide more information than simple species richness. Using functional attributes leads to a more stable approach and offers the possibility of being standardised across regions with different species (Seymour et al. 2016). However, traits vary as a response to environmental factors, which stresses the need for reference models to accurately estimate the contribution of human-induced stressors. Furthermore, evidence for sensitivity to several stressors has been found (Menezes et al. 2010), but more research is necessary to effectively link community level response to environmental and anthropogenic stressors at a global scale (Demars et al. 2012). Due to the endless possibilities functional traits entail, there is a need to develop a clear definition of these traits and to assign them to species. Numerous ways have been developed to allocate species to certain groups varying from indices based on sensitivity (e.g. SPEAR index, which considers sensitive species by a combination of specific traits including aspects of physiology, life cycle or behaviour) to divisions based on functional feeding groups. Whatever functional attribute is used to classify invertebrate groups, the trait-based method does not require taxonomic expertise.

2.1.1.3 Interaction-based indicators

Ecosystem processes and services depend on interactions between individuals and biodiversity, ecosystem functioning and a systems sensitivity to environmental change can be affected by these interactions (Tylianakis et al. 2007; Thompson et al. 2012). Even though interactions are crucial in a community, traditional freshwater monitoring does not consider them, with the risk of missing certain effects. Furthermore, effects that could not be explained by traditional monitoring were resolved when taking in account species interactions within a food web (Layer et al. 2011). A network-based approach addresses both structural and functional metrics, because of the linkage between structural metrics and functioning, thus offering more complete information on the community as a whole.

Interaction-based metrics can reveal effects of multiple, both direct and indirect stressors on a community, but which metrics are most ecologically informative remains to be investigated. Several studies show that the suitability of interaction-based metrics for biomonitoring is variable (e.g. Heleno et al. 2012; Layer et al. 2011). Also, gathering data on interactions is currently too labour intensive. However, current ecological monitoring data can be used to infer information about interactions in such a way that each network does not have to be constructed anew from direct observations. The ecological network approach, in the future possibly enhanced by molecular techniques, can provide the link between structure and functioning that is currently missing (Gray et al. 2014).

2.1.2 eDNA

By interacting with their surroundings, organisms expel DNA to the environment. This environmental DNA (eDNA) originates from cells, organismal excretions and dead animals

and can be used to determine whether a species is present. It has potential to quantify species diversity and thus to monitor biological quality elements in aquatic environments.

eDNA is a new technique still in its infancies and many studies are conducted to investigate the robustness of this approach. Whereas traditional community sampling, based on visual detection and counting, is labour intensive, slow and often invasive, eDNA-based sampling can be a rapid, non-invasive way of monitoring species. This approach is proved to be superior to visual detection (Dejean et al. 2012; Takahara et al. 2013). In combination with next generation sequencing (NGS) a single, standardised water sample has the potential to provide information about entire communities across taxonomic groups. This information can resolve ecological networks (Vacher et al. 2016), generating a series of network properties (e.g. connectance) and creating an standardised set of measures for monitoring that does not require taxonomic expertise. eDNA is not only applicable to diversity measurements of fish and macro-invertebrate species under focus in existing monitoring schemes, but also has the potential to provide a standardised method across a taxonomically diverse set of organisms, including bacteria and fungi, who are currently overlooked.

Although numerous studies are conducted in the field of eDNA, there are still several problems to overcome before eDNA is ready for implementation in water quality monitoring programs. Even though this tool can identify species and is able to provide detailed information on species richness, the ability to provide biomass estimates is currently limited (Leese et al. 2016). Additionally, long-distance transport of eDNA remains a problem, especially in flowing waters, since this results in variable spatial scales. Several methodological pitfalls associated with eDNA are the possibility to obtain false positive results through contamination, inhibition by humic substances, sequence errors, the need for reliable reference DNA-sequence databases and certain interpretational problems such as the inability to distinguish living versus dead individuals and particular life stages (Thomsen & Willerslev 2015).

The fact that eDNA is ubiquitous, is produced by all taxa and that the sampling methods do not involve capturing organisms and can easily be standardised, makes this an emergent research field (Bohmann et al. 2014). With numerous studies being conducted in this field, analysis of environmental DNA is becoming an effective means of water quality assessment. Particularly, the use of eDNA for estimates of fish diversity/richness is thoroughly studied and suited for short term implementation (Thomsen et al. 2012a; Thomsen et al. 2012b). However, in the case of other taxa additional evidence is required for eDNA to move from fundamental research to an applied monitoring tool within water quality monitoring programs.

2.1.3 Remote sensing

The earth's surface absorbs and reflects energy and these rates vary because of photons interacting differently with structures at different wavelengths, resulting in varying levels of reflected and emitted energy. Using the principles of spectroscopy, remote sensing measures the reflected energy through high resolution imaging, determining the composition of the earth's surface. Depending on the aim of the study, sensors are attached to either airborne devices, such as satellites or drones, or to ground based platforms.

Currently, remote sensing is used for assessment of terrestrial habitat quality and for supporting management (Spanhove et al. 2012; Kachelriess et al. 2014). A major advantage of this technique in water quality monitoring is the ability to rapidly screen large areas and detect water quality variables from a distance. However, for calibration and validation additional in situ data is required. Satellites can provide integrative, frequent and consistent remote sensed data on a national scale. Several drawbacks associated with space platforms are the costs, limited temporal resolution, the problem of cloud cover and the enormous amount of data generated which requires advanced computers and personnel. Also, the ability of satellites to assess small water bodies is limited by spatial resolution. Drones on the other hand appear to be a promising tool for monitoring water bodies on regional scale. Nonetheless, identifying species from images remains challenging.

In the context of water quality assessment remote sensing is used to detect mainly structural water quality variables ranging from ecological parameters, like macrophyte abundance and cyanobacterial biomass, to physicochemical parameters, like non-algal particulate matter, depth and turbidity (Dekker & Hestir 2012). Remote sensing for water quality monitoring is gaining importance (Dörnhöfer & Oppelt 2016). Recently, several European projects were set up (e.g. INFORM and EOMORES) to develop remote sensing technologies for water quality assessment. In a few years, this probably will result in promising techniques to aid the water quality assessment of European waters.

Functional indicators

Present ecological monitoring is based mostly on structural metrics like diversity and abundance of target species, while neglecting ecosystem functioning. However, many ecosystem processes are not necessarily linked to structure, e.g. rates of respiration cannot be determined through diversity measurements of macroinvertebrate communities. Therefore, process-based indicators for water quality assessment are promising, since they provide ecologically relevant information, include different organizational levels, enable spatial and temporal comparison and do not require taxonomic expertise. It has become increasingly apparent that functional metrics should be implemented in water quality monitoring programs and that they, in combination with structural metrics, provide a more holistic picture of the status of a water body.

Several processes (e.g. microbial respiration and rates of nutrient uptake) can aid in water quality assessment (von Schiller 2016), although two processes receive a significant amount of attention in the scientific world due to their sensitivity and simplicity: decomposition and ecosystem metabolism.

2.2.1 Whole ecosystem metabolism

Whole ecosystem metabolism is a measure of how much total organic carbon is produced (gross primary production, GPP) and consumed (ecosystem respiration, ER) and assesses the balance between energy supply and demand within an ecosystem. This approach provides information on relative importance of key sources of energy in freshwater ecosystems. The

direct measurement of the food base helps to determine the life supporting capacity of aquatic systems. Like decomposition, ecosystem metabolism is affected by a wide array of factors ranging from naturally varying components to elements influenced by anthropogenic pressures.

Ecosystem metabolism is determined by measuring changes in either O_2 or CO_2. Since dissolved O_2 is relatively easy to measure and has a high magnitude of change, this means of ecosystem metabolism assessment is preferred over CO_2 measurements. One or two data-logging O_2 meters can provide information representative for the entire reach within two days (Young et al. 2008). Unlike decomposition rates, equipment for metabolism measurements is pretty expensive and must stay on-site 24h a day. Also, estimation of reaeration (i.e. the amount of O_2 diffusing between air and water) is required and effects of natural variability must be taken into account when interpreting results. Lastly, determination of ecosystem metabolism is not possible in small, turbulent streams with low productivity.

Even though interpretation of ecosystem metabolism rates requires further investigation in order to provide reliable information on water quality, the ecosystem metabolism approach can readily be implemented in current monitoring programs, especially on sites with a continuous monitoring platform already present.

2.2.2 Decomposition

Leaf litter degradation is a fundamental process in carbon and nutrient cycling of freshwater ecosystems (Wallace 1997; Odum & de la Cruz 1963) and is influenced by both abiotic and biotic factors such as ultraviolet radiation, litter quality and composition of detritivorous communities (Throop & Archer 2009).

There are a few challenges involved in the interpretation of decomposition parameters. Breakdown rates differ between ecoregions and are influenced differently by environmental factors and abundance, diversity and distribution of decomposing organisms, which makes it difficult to assess the impact of human-induced stressors alone. Therefore, translating substrate mass loss into valuable information for water quality assessment requires additional evidence.

Several sampling methods have been developed in order to measure decomposition in aquatic environments. To assess breakdown rates most methods simply measure mass loss of a substrate over time making these tools inexpensive, easy to use and highly standardised.

Leaf litter originating from riparian vegetation is the major source of organic matter (OM) in aquatic systems (Wallace et al. 1999). By utilising natural leaf litter as a substrate, the litterbag approach has high ecological relevance. The downside leaf litter is its natural variability between geographic regions, even within species (Lecerf & Chauvet 2008), which impedes spatial comparison. Also, the fragility of leaf litter can result in unwanted losses of litter bag contents.

Commercially available teabags are not bothered by unwanted litter loss, but still cope with the spatial comparison constraint. This approach is currently deployed in citizen science projects (e.g. NETLAKE) because it is cheap, standardised, environmentally realistic and easy to use. The downside of commercially available teabags is the fine mesh size and thus the exclusion of macroinvertebrates.

Both DECOTABs, cotton strip assays (CSA) and wood make use of standardised substrates and therefore enable spatial comparison between geographical regions. Standardised pieces of wood have the advantage of adjustable incubation time through varying wood size and shape, but it is unknown to which extent the decomposition of wood relies on xylophagous invertebrates.

CSA determines loss of tensile strength of standardised cotton fabric rather than mass loss. This cellulose-based tool requires less incubation time than leaf litter. Even though CSA uses cellulose, a compound abundant in ecosystems, as a substrate, it is not as attractive to macroinvertebrates as leaf litter (Tiegs et al. 2013). Also, the simplicity of the substrate makes this method sensitive to physical abrasion and fragmentation.

DECOTABs are less susceptible to fragmentation and provide a measure of both microbial decomposition and macroinvertebrate consumption. These agar-based disks are inexpensive, easy to prepare and deploy and can be adjusted to suit the needs of the research in question by adding the desired substrate, such as cellulose or leaf litter in the case of water quality assessment. The main disadvantage of the DECOTAB is its texture, that does not resemble natural leaf litter. As a consequence, the extent to which DECOTABs reflect decomposition remains to be tested.

Overall, its sensitivity to anthropogenic stressors and the simplicity of measurements make decomposition suitable for monitoring purposes (Young et al. 2008). When interpretational constraints have been overcome, decomposition can act as an important means for determination of human impacts on freshwater ecosystems. It should be noted that many tools aiming to study decomposition and consumption rates currently rely on standardized material that is often composed of alien organic matter. This is likely the primary source of variation that currently seems to prevent linkages between ecosystem health and ecosystem processes since organic matter composition is of prime importance for invertebrate consumption and diversity (Lecerf and Chauvet, 2008; Hunting et al., 2013). Incorporation of organic matter originating from the site of interest seems to provide a solution to overcome this variability (Hunting et al., 2016).

Citizen science networks

Science projects involving citizens are gaining popularity and lately various projects on environmental research, such as Riverflies or NETLAKE, have appeared. Under the guidance of a scientist or research groups, citizen scientists voluntarily collect or process data in projects on for instance conservation biology or ecological restoration. Part of its popularity can be assigned to the existence of easily available technical tools like the internet and smartphones (Silvertown 2009). Scientists increasingly realize that citizens are a free source of labor and skill, which makes citizen science networks a cost-effective means of gathering data.

Clearly, citizen science projects can only cover relatively straightforward tasks that can easily be standardized like decomposition measurements or simple invertebrate monitoring and data collected by these projects must be validated by an expert. However, citizen science networks are largely self-organizing and entail comparatively little costs

through the use of volunteers (Jackson et al. 2016). Besides generating knowledge on ecosystem health, citizen science networks have the additional advantages of educating citizens and raising awareness on the importance of water quality monitoring.

Ecological Key Factors (ESF)

Stichting Toegepast Onderzoek Waterbeheer (STOWA) has developed a framework of Ecological Key Factors (in Dutch: Ecologische Sleutelfactoren; ESF) to assist water managers in the classification of ecological status of a water body. This decision support system aims to acquire insight in ecosystem functioning and to improve ecology and water quality through understanding of aquatic systems. The understanding of aquatic ecosystems is essential for the development of appropriate measures to improve water quality. The determination of ecological status within the ESF approach is based on nine different factors, each of which forms a threshold for a properly functioning aquatic system. A water body receives either a green or a red status for each ESF and it is clearly indicated where additional measures have to be implemented.

Factors are categorized in differently weighed groups. Water productivity (ESF1), light (ESF2) and sediment productivity (ESF3) are conditions for the recovery of submerged vegetation and form the basis of a healthy ecosystem. Habitat suitability (ESF4), connectivity (ESF5) and Removal (ESF6) are additional conditions for the recovery of species and communities. Organic pollution (ESF7) and toxicity (ESF8) are environmental factors with a dominant function. If one of these factors receives a red status, it becomes prioritized. Lastly, context (SF9) addresses questions at a higher level: for instance by assessing how we can improve an aquatic system and what appropriate measures should be taken. ESF are applicable for (nearly) stagnant waters by the end of 2017 and ESF for streams are currently under development.

Effect-based tools

The list of emerging substances in our aquatic environment is ever growing. It is unmanageable to derive an Environmental Quality Standard (EQS) for every single pollutant that enters the aquatic environment. Furthermore, on the basis of analysed concentrations alone, effects of pollutants on the environment (e.g. antagonistic and synergistic effects) cannot be adequately estimated. To overcome these difficulties, a novel way of monitoring has to be developed.

Effect-based tools are based on biological components to adequately assess the effect of pollutants and provide an integrated picture of overall impact on the aquatic environment. Effect-based tools can target different levels of organisation, from cells and tissues to whole organisms and even communities. Tests can be performed under laboratory conditions (in vitro and in vivo bioassays) or in the field (e.g. biomarkers and in situ bioassays) and usually target different levels of organisation, from cells and tissues to whole organisms and even communities. For chemical concentration data to be valuable to environmental impact assessment effects of pollutants and their modes of action must be known. This way, effect-

based tools can complement current water quality monitoring by bridging ecological and chemical status. Effect-based tools covers mixture effects and effects of compounds that are not mentioned on the priority substances list and comprise a wide variety of toxicity mechanisms in various organisms (Di Paolo et al. 2016).

3.1 Biosensors

A biosensor is generally defined as an analytical device that combines a biological recognition element, the bioreceptor (e.g. enzymes, antibodies, DNA or microorganisms) with a physical transducer (e.g. electrochemical, thermal, optical or piezoelectric), immobilising the bioreceptor and converting a biological response to a quantifiable signal proportional to the analyte concentration (Lagarde & Jaffrezic-Renault 2011; Grieshaber et al. 2008). In this way, biosensors can detect compounds, groups of compounds or effects either through single sample field screening or continuous monitoring.

The possibility of miniaturization and portability and the potential to measure pollutants with minimal sample preparation, makes the biosensor an appropriate method for in-situ analysis (Rodriguez-Mozaz et al. 2006). However, current sensors are only used in laboratory settings and the majority of developed biosensors require additional verification and validation before commercialisation. Overall, the environmental application of biosensors in water quality assessment is very promising in terms of sensitivity, selectivity and potential for field use, but more evidence is required before implementation in the field.

3.2 Biomarkers

Biomarkers are indicators for pollutant stress that can be related to exposure to environmental chemicals. In the biomarker approach, alterations at the sub-individual level (e.g. molecular, cellular, biochemical and physiological) caused by external stressors are assessed (Connon et al. 2012). Like in biota chemical monitoring, cells and tissues of wild organisms are directly analyzed. However, biomarkers measure sub lethal responses to pollutants rather than pollutant concentrations. On the one hand, biomarkers of exposure indicate internal exposure of pollutants concentrations and can be used to screen specific groups of chemicals. Biomarkers of effect on the other hand indicate functional changes at the organismal level. They can discover effects at individual level before effects at the level of the population occur and therefore exert an important early warning function (Connon et al. 2012).

Usually, biomarkers are very specific and thus have a low overall human impact detection potential. However, it does allow the detection of specific stressor-effect relationships, enabling discrimination between impacts. The use of field-exposed organisms increases ecological relevance and analytic costs are relatively low (Wernersson 2016). Difficulties are involved in the upscaling of biomarker responses. Due to compensatory mechanisms and other factors, responses at individual level do not automatically imply responses at the community or ecosystem level (Bonada et al. 2006).

Several biomarkers are well described in scientific literature and few of them are included in environmental monitoring programs (Wernersson et al. 2014). However, more

evidence on biomarkers is needed to understand the effects of pollutants and naturally occurring factors.

3.3 Bioassays

Bioassays are a rapid and sensitive detection of pollutant effects in biological systems, either at the cellular level or at the whole organism level. Even though many pollutant effects are not lethal, they do have the ability to negatively affect the fitness of individual organisms or populations.

In vitro bioassays offer a rapid and sensitive detection of chemical activity and combined effects of chemical substances with similar modes of action at cellular level (Connon et al. 2012). In vitro bioassays are highly sensitive and respond to many substances while having a relatively short exposure time. Through integration of the effect of all substances with the same mode of action, in vitro bioassays can be used to quantify and distinguish antagonistic effects (Wernersson et al. 2015). However, these assays are highly simplified, do not cover the complexity of an organism and the predictive power for effects at higher organizational levels is limited.

In vivo bioassays use whole organisms to measure potential biological impact of pollutants. It allows quantification of toxic effects while excluding effects of other environmental stressors. In vivo bioassay is a broad-spectrum assay: it responds to a wide variety of substances. The exposure time is relatively long, especially at low concentrations of pollutants. In vivo bioassays often use model species. While model species provide a standardised, controlled means of toxicity testing, the results may not be representative for all species and do not include other environmental stressors or variable exposure (Connon et al. 2012). In-situ testing on the other hand is more ecologically relevant, but is disturbed by stressors that organisms encounter in a natural environment. Using species of different trophic levels simultaneously, a battery of bioassays mimics a simple food chain, integrating interactions between species, such as competition and predation.

Simultaneous acting of unknown chemicals and stressors can result in much higher toxicity than predicted from analytical measurements. Therefore, combined with chemical analysis, bioassays provide a more complete framework for water quality assessment.

3.4 Biological Early Warning System

Whole-organism bioassays and cell-based biosensors can be applied to obtain accurate real-time data that can serve as an early warning. These Biological Early Warning Systems (BEWS) can detect a wide range of pollutants within a short time frame and enable water managers to take appropriate measures at an early stage. Whereas the sensors of continuous monitoring stations are non-living devices, the sensors in a BEWS are living organisms, such as bacteria, algae, bivalves, fish or a combination. The Mosselmonitor (http://www.mossel-monitor.nl/) for example, is based on the behaviour of mussels, issuing an alarm when shell movements of mussels deviate from their normal pattern. The unattended operation and low maintenance makes this system cost-effective compared to standard physicochemical monitoring systems. However, behaviours are non-linear which makes it difficult to objectively quantify and interpret behavioural data (Bae & Park 2014). Moreover, this form of

continuous monitoring generates large amounts of data and even though organisms respond to a multitude of chemicals, they cannot provide qualitative or quantitative information about chemicals.

3.5 Pollutant identification

Effect based tools indicate hazards induced by pollutants and provide information on toxicological endpoints. However, more information is needed to identify pollutants and to link exposure to effects. By combining biotesting, fractionation, chemical analysis and confirmation processes, pollutant identification approaches identify chemical compounds from environmental samples that cause a biological response. Even though the testing procedure is mostly the same, both approaches have a different theory.

Effect directed analysis (EDA) is based on the fact that samples can contain thousands of organic chemicals of which only a fraction can be analysed by chemical target analysis. In order to identify pollutants, bioassays are used on an extracted and pre-concentrated version of the sample to quantify effects (Burgess et al. 2013). If a significant effect has been found, the complexity of the sample is reduced through fractionation, eliminating fractions with no biological activity. Finally, the identified pollutants are confirmed as the cause of the measured effect (Brack et al. 2016). This iterative process harnesses biological effects as the basis to narrow down possibilities and aims to direct chemical analysis to the compounds that contribute the most. Any endpoint that can be perceived and measured with sufficient throughput can be used as a basis for EDA.

Toxicity Identification Evaluation (TIE) aims to answer the question whether an effluent causes adverse effects on aquatic organisms when emitted to the environment. With ecological relevance as primary goal, TIE applies in vivo bioassays to detect potentially toxic chemicals and focuses on characterization of unmodified samples, thus avoiding extraction and pre-concentrations steps as far as possible. Like EDA, TIE requires a confirmation step to validate that the observed effects can be explained by the identified pollutants. The TIE approach is highly specific for classes of pollutants, moderate for specific pollutants and only includes acute toxicity. However, the absence of acute toxicity does not indicate that there is no effect of pollutants on a community.

Since pollutant identification processes can be very costly, several ideas have been proposed that use a tiered approach. First of all, chemical analysis provides an indication of which chemicals are present. In the second tier chemical monitoring data is complemented with toxicological data through the use of bioassays. Lastly, when there is a significant risk which cannot be explained by chemical monitoring data, EDA can be used to unravel toxicity (Brack et al. 2016). In order to diagnose the cause of observed toxicity both EDA and TIE can be useful in water quality assessment, either to identify emerging compounds or to assess regulated substances.

Appendix II. Authorization of chemicals versus Water Framework Directive

There are many crucial differences that can be identified between prospective risk assessment within chemicals authorization and retrospective chemical and ecological monitoring. Those crucial differences can be enlisted in classed as follows:

1. Deviating environmental quality criteria

   Ecological recovery is sometimes considered within the authorization of chemicals versus the " no effect" quality criteria in the WFD. On top of that the authorization criteria considers more elements that (eco)toxicity in their decisions giving that already the majority of pesticides for instance have a lower WDF quality criteria compared to the authorization criterion. The moment the substances are allowed on the market they thus can have a high hazard ratio resulting in higher predicted exposure levels compared to the predicted no effect concentrations at certain times of usage.

2. Deviating evaluations

   The single substance authorization versus the ecological water quality monitoring that considers by definition joint impacts of chemical mixtures and natural stressors. The authorization based on model assessments (prospective assessments) versus retrospective assessment within the WFD based on actual monitoring samplings and analysis. Most issues under debate within this arena are the assumption of the models to work with 100% Good Agricultural Practice, which is known to deviate between types of substances used e.g. a factor 10 % above GAP for herbicides usage and 50% above GAP for insecticides usage (De Snoo, 2003), and for sectors in which chemicals are applied - (emissions underestimated up to 10-100 times). Similarly many debate is on the usage instructions and their practicalities in usage versus the enforceability when these instructions are neglected.

3. Deviating spatial resolutions

   The prospective risk assessment of e.g. pesticides focusses on the emissions in ditches adjacent to agricultural fields which are mostly small and shallow. While the WFD monitoring mainly focusses on larger watersheds to which many agricultural ditches are connected. Evidently, these water bodies differ in n umber of aspects, including water volume, dilution and mixture factor, as well as time necessary to degrade substances (due to differences in e.g. photodegradation).

4. Deviating ecological endpoints

   The ecological species selected in the regulatory base set are model test species that are typically well-studied organisms that have " easy-to-culture or maintain" characteristics allowing for the laboratory-driven effect tests. These organisms not necessarily are the most vulnerable species as identified based on mode-of-action – which was illustrated within the insecticides research and policy actions around imidaclorpid as that chemical was initially authorized based on ecotoxicity data performed with the standard species set that lacked an insect (Vijver et al 2013; Roessink et al., 2103; Vijver et al 2017).

In line with the same mismatch between the regulatory species base versus the most vulnerable species in an ecosystem, is the example that some ecological important groups such as fungi is totally ignored within most cases of the authorization. The lower tiered fate and ecotoxicity tests within the authorization procedure report mostly well-defined acute and sub-lethal endpoints. However, these endpoints might not always be the endpoints of interest for chemicals (e.g. with specific Mode of Actions, metabolites) affecting the fitness of species or indirect effects on multiple species and their ecological functioning.