Collaborative Project (large-scale integrating project)
Grant Agreement 226273
Theme 6: Environment (including Climate Change)
Duration: March 1st, 2009 – February 29th, 2012

WISER

Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery

DELIVERABLE

Deliverable D3.1-5: Integrated assessment of lake phytoplankton

Lead contractor: **Natural Environment Research Council (NERC)**

Contributors: **Carvalho L., Poikane S., Lyche Solheim A., Phillips G., Borics G., Catalan J., De Hoyos C., Drakare S., Dudley B., Jarvinen M., Laplace-Treyture C., Maileht K., McDonald C., Mischke U., Moe J., Morabito G., Nõges P., Nõges T., Ott I., Pasztaleniec, A., Skjelbred B. & Thackeray S.**

Due date of deliverable: **Month 36 (February 2012)**
Actual submission date: **Month 37 (March 2012)**

Project co-funded by the European Commission within the Seventh Framework Programme (2007-2013) Dissemination Level		
PU	Public	X
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Content

Non-technical summary

Phytoplankton constitute a diverse array of algae that live suspended in the water column of lakes and reservoirs. They are short-lived organisms (generation times of days to weeks) and they derive their nutrients exclusively from the water column. These features make this biological quality element the most direct and earliest indicator of the impacts of changing nutrient conditions on lake ecosystems. It also makes them particularly suitable for measuring the success of restoration measures following reductions in nutrient loads. This report summarises the work on lake phytoplankton in the EC WISER Project. It summarises a number of measures, or metrics, developed in WISER for using phytoplankton to assess the ecological health of European lakes, as required for the Water Framework Directive It also reviews metrics developed by Member States. It examines the strength of these metrics, specifically in relation to representing the impacts of eutrophication pressure. The report also examines how these measures vary naturally at different locations within a lake, as well as between lakes, and how much variability is associated with different replicate samples, different months within a year and between years. On the basis of all this analysis, three of the best metrics (chlorophyll, PTI & cyanobacterial biovolume) are recommended for use in the WFD Intercalibration process, or for adoption as national metrics by member states. The final discussion examines whether these metrics effectively represent the impact of eutrophication on the structure and functioning of lake ecosystems.

Introduction

The phytoplankton community forms a key component of primary production in lakes. The fact that phytoplankton are short-lived and derive their nutrients from the water column makes this biological quality element the most direct and earliest indicator of the impacts of changing nutrient conditions on lake ecosystems (Lyche-Solheim, this issue). There are numerous socio-economic problems associated with eutrophication-related increases in phytoplankton abundance, particularly with increasing frequency and intensity of toxic cyanobacteria blooms. These include detrimental effects on drinking water quality, filtration costs for water supply, recreational activities, and conservation status. Phytoplankton are, therefore, a key indicator of the health and functioning of freshwaters in relation to eutrophication pressure, and for measuring the success of restoration measures following reductions in nutrient loads. The EC Water Framework Directive (EC, 2000) requires the ecological status of lakes to be assessed on the condition of their biological quality elements (Article 8, annex V). Annex V of the WFD specifically outlines three features of the phytoplankton quality element that need to be considered in this assessment for lakes:

1. Phytoplankton biomass or abundance and its effect on transparency conditions
2. Phytoplankton composition
3. Planktonic bloom frequency and intensity

Here we briefly review these three features, but focus particularly on metrics for the latter two, which required specific further developments for the WFD. As part of this, we review national metrics that have been developed for lake phytoplankton for the WFD. We then summarise sources of uncertainty in all three features based on analyses carried out for the WISER Project. We recommend which metrics are most suitable for WFD assessment and the minimum sampling requirements for robust ecological assessment. Finally we discuss the gaps in current assessment, particularly in relation to lake functioning and cross-BQE measures of eutrophication pressure

Biomass, abundance and transparency

In general, as nutrient concentrations increase, phytoplankton biomass or abundance shows more frequent and sustained peaks throughout summer and transparency declines. There are some specific exceptions to this, such as shallow macrophyte-dominated lakes, which are highlighted later in the discussion of the need for a more holistic approach to ecological assessment in relation to eutrophication pressures. Phytoplankton biomass or abundance is generally measured as "biovolume". Alternatively, concentrations of the photosynthetic pigment chlorophyll *a* are used as an approximate measure, widely adopted in European and international lake monitoring and classification schemes. Measurements of chlorophyll *a* can be problematic in that concentrations vary depending on algal composition and their physiological state (Reynolds 1984). In general, cyanobacteria have less chlorophyll *a* per unit biomass than Chlorophyta. Direct counts and measurements of algal biovolume are potentially, therefore, a more accurate measure of phytoplankton biomass or abundance. Biovolume measurements are, however, much more time-consuming to make and often more prone to errors between different analysts (see later section on uncertainty).

One of the first classification schemes developed for phytoplankton abundance was that of Carlson (1977) who used chlorophyll *a* (and secchi disc depth) as a measure of "trophic status". The most widely recognised classification in terms of chlorophyll *a* is, however, that developed during the OECD programme on eutrophication (OECD, 1982). This developed quantitative regression models relating chlorophyll a concentrations to total phosphorus concentrations and outlined chlorophyll standards for different trophic classes (oligotrophic, mesotrophic and eutrophic) based on expert opinion. More recently reference-based classification schemes for chlorophyll *a* have been developed in individual Member States specifically for the WFD (e.g. Carvalho et al., 2006; Sondergaard et al. 2005) and chlorophyll a has been successfully Intercalibrated to ensure standardised quality classes exist across regions of Europe (Poikane et al., 2010). For this reason the WISER Project did not re-visit assessment schemes for phytoplankton biovolume or chlorophyll a. It has, however, examined sources of uncertainty in their measurement and on the basis of this provided recommendations for WFD sampling programmes (see Section X).

Composition

Most algal classes are found in lakes spanning the entire nutrient gradient. The only exceptions to this are chrysophyte algae that are characteristic of more nutrient poor (and acid) waters. Compositional changes due to nutrient enrichment become more apparent at the generic and species level. For example, of the diatoms, *Cyclotella* species are frequently associated with nutrient poor lakes and *Stephanodiscus* species tend to dominate following enrichment (Bennion 1994; Wunsam & Schmidt, 1995). Cyanobacteria, such as the large colonial and filamentous genera *Microcystis*, *Aphanizomenon* and *Anabaena* also tend to increase in abundance in response to increasing nutrient concentrations (Reynolds 1984). The philosophy of ecological status in the WFD is that it is "an expression of the quality of the structure and functioning of the system". Phytoplankton compositional responses to eutrophication can also be considered in terms of functional groups (Reynolds et al., 2002). Trait-based, functional classifications are increasingly being used in ecology because of their connection with ecosystem functioning. Among functional traits, cell size is a key feature, being related to the efficiency of many eco-physiological processes (nutrient assimilation, photosynthetic efficiency, respiration, buoyancy), most of which are affected in some way by nutrient changes (Capblanq & Catalan, 1994). Following this approach, a phytoplankton assemblage can be described in terms of size spectra (Kamenir & Morabito, 2009) or Morpho-Functional Groups (Reynolds et al., 2002; Salmaso & Padisak, 2007).

In recent years a large number of national assessment systems for phytoplankton composition have been under development for the WFD, including taxonomic and functional approaches (Poikane 2009). One of the key actions identified by the WFD is to carry out a European benchmarking or intercalibration (IC) exercise to ensure that these assessment systems are comparable and, in particular, that good ecological status represents the same level of ecological quality everywhere in Europe (EC, 2000, Annex V). In this paper, we review the national metrics submitted by the end of the 2nd phase of the Intercalibration process (November 2011) and outline three compositional metrics, developed in WISER, for potential use as a "common metric", a common measurement scale for comparison of national metrics in the IC process. These three WISER composition metrics are:

1. Phytoplankton Trophic Index (PTI) – a taxonomic-based sensitivity index
2. Size Phytoplankton Index (SPI), based on size classes
3. Morpho-Functional Group Index (MFGI) – a combination of size and functional group

Bloom frequency and intensity

There is no consistent agreement on a definition of a phytoplankton bloom, although it is always used in relation to an abundant crop of a particular class of algae. Annex V of the WFD indicates that a bloom metric should incorporate some measure of both bloom intensity (spot measures of magnitude/abundance) and how frequently they occur (or potentially could occur) over a particular specified time period (e.g. frequency within a summer period or frequency over the 6 year WFD reporting period). The term "bloom" has been associated with surface scums of cyanobacteria for hundreds of years (McGowan & Moss, 1999). Cyanobacteria are widely recognised to increase in dominance and abundance in response to increasing nutrient concentrations, often resulting in dense, mono-specific blooms during summer in eutrophic waters (Reynolds 1984). More recently, lake ecologists have used the term to refer to spring and autumn increases in diatoms and marine biologists have referred to blooms of diatoms or dinoflagellates. Annex V of the WFD characterises moderate status lakes as those in which "persistent phytoplankton blooms" may occur during summer months and, for this reason, almost certainly had in mind summer blooms of cyanobacteria. Mischke et al. (2011) proposed three characteristics of a summer phytoplankton bloom in lakes:

- High phytoplankton abundance
- Uneven community – dominance by one type of algae, usually one or two species
- Abundance of nuisance species e.g. potentially toxic cyanobacteria

With these characteristics in mind, WISER developed two potential bloom metrics to test and consider for IC purposes:

1. Pielou's Evenness Index (J) (incorporating a critical abundance threshold)
2. cyanobacterial abundance (actual biovolume – not relative % abundance)

Aims of this paper

1. To review national metrics developed as part of the IC process
2. To summarise the strength of WISER composition and bloom metrics
3. To provide an overview of uncertainty in WISER metrics and chlorophyll a and use the results to recommend guidance on sampling and analysis
4. To review application in Intercalibration and recommend metric combinations for whole phytoplankton BQE assessment for the WFD
5. To identify gaps for future work

Methods

Review of national metrics

National metrics, submitted in GIG Milestone reports, were reviewed for Intercalibration (Poikane, 2012). Based on existing metric classifications (Karr and Chu 1999, Hering et al. 2006), we grouped metrics into the following types: (1) abundance metrics (e.g., chlorophyll-a and total biovolume), (2) composition metrics (e.g., % of Cyanobacteria), (3) sensitivity/tolerance metrics (e.g., trophic indices) and (4) richness/diversity metrics (e.g., evenness or diversity indices). Note that composition metrics largely overlap with sensitivity / tolerance metrics as % or biovolume of Cyanobacteria is often used as a sensitivity metric

Strength of composition and bloom metrics

The sensitivity of the WISER phytoplankton metrics to eutrophication pressure was assessed from regression analyses of dose-response curves along TP gradients using large scale pan-European datasets from >1500 lakes from 21 countries. Full details of the methods used are provided in Phillips et al. (2010) and Mischke et al. (2011).

Uncertainty and Sampling Guidance

Spatial and analytical sources of variability of a number of phytoplankton metrics was assessed using WISER data sampled in 25 lakes in 2009.

Results

Review of national metrics

Most of the national methods for the phytoplankton BQE comprise either 2 metrics (one of them related to phytoplankton biomass, another to taxonomic composition) or 4 - 5 metrics (including several parameters both for biomass and species composition) (Figure 1). Only one method contains just one metric (SE method for acidification).

Table 1. Overview of the metrics used in MS phytoplankton assessment methods

Metric type	Metric	Number
Biomass metrics		**40**
	Chlorophyll-a	23
	Phytoplankton biovolume	13
	Average of chlorophyll-a and biovolume	3
	Secchi depth	1
Sensitivity / tolerance metrics		**23**
	Indices based on indicator species	13
	Indices based on taxonomic groups	8
	Indices based on indicator values of functional groups	2
Composition metrics		**17**
	Relative abundance of Cyanobacteria	9
	Biovolume of Cyanobacteria	6
	Relative abundance of other algal groups	2
Richness / diversity metrics		**7**
	Evenness index	2
	Taxa richness	2
	Diversity index	3
Total		**87**

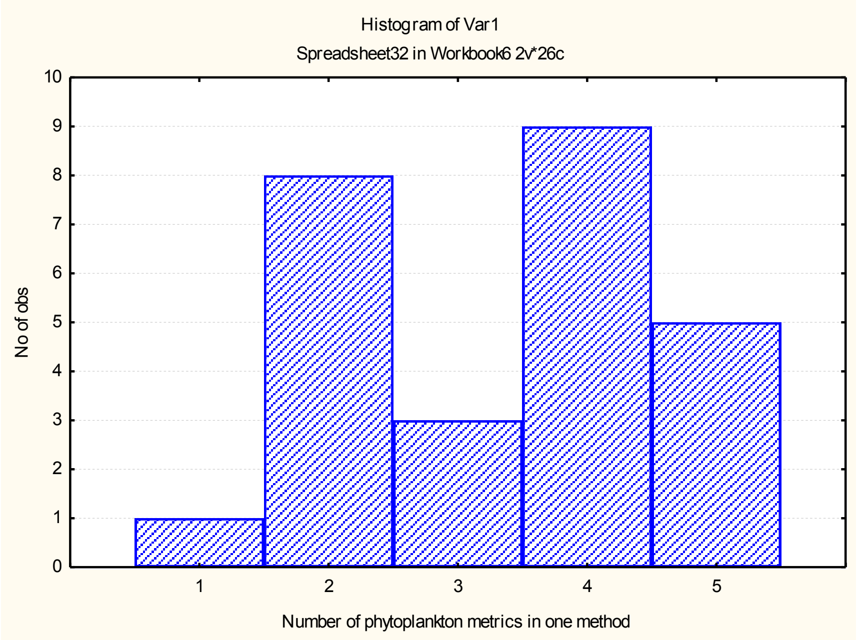

Figure 1: Number of individual metrics combined to assess the phytoplankton BQE.

Almost half of the metrics characterise phytoplankton abundance (46 %), while another half were divided between sensitivity / tolerance metrics (26%) and composition metrics (20%) (Table 1). Richness/diversity metrics were rarely used (7 %) and no national metrics were specifically termed “bloom” metrics, although 15 of the 17 composition metrics were based on the relative or absolute abundance of cyanobacteria (Table 1). Note that composition metrics largely overlap with sensitivity metrics as one of the composition metrics, in particular the relative or absolute abundance of Cyanobacteria is often used as a sensitivity metric, as it is a well-known indicator of eutrophication.

The most frequently used biomass metric is chlorophyll -a (23 metrics), used alone or together with total biovolume. Almost all MS included some version of sensitivity / tolerance metrics where 3 patterns can be distinguished: (1) The most frequent sensitivity indices are based on indicator taxa lists and their trophic scores and weighting factors (e.g., Brettum 1989, Dokulil 2005, Salmaso 2006), (2) other indices were based on biovolume of a given algal group, or on the ratios between the biovolumes of several algal groups (Catalan, 2003, Nygaard 1949, adapted by Ott 1995); 3) only 2 MS used indices based on a functional group approach (Reynolds et al 1989) where indicator values were assigned to each group (Padisák et al. 2006).

Composition metrics were largely based on relative or absolute abundance of cyanobacteria (9 and 6 metrics, respectively); only in a few cases were other algal groups (Chrysophyta and/or Bacillariohyta) used. Richness/diversity metrics were included in only 3 MS methods, represented by evenness, taxa richness or diversity indices.

Strength of composition and bloom metrics

The best phytoplankton metrics in terms of regression strength with TP was the PTI ($r^2 = 0.67$), and chlorophyll a ($r^2 = 0.63$, for lakes with TP<100µg/l) (Phillips et al., 2010; Mischke et al., 2011). The weakest relationships were generally found for the evenness metric, although the strength of the SPI and MFGI with TP varied greatly between GIGs (Table 2).

Table 2. Overview of metric sensitivity to pressure for biological quality elements in lakes. GIG = Geographical Intercalibration Group. CB = Central European and Baltic region, N = Northern region, M = Mediterranean region. Data taken from Phillips et al. (2010) and Mischke et al. (2011).

Metric	Metric description	Pressure	r^2	GIG	p	N
Chla	Chlorophyll a (µg/l)	Eutrophication (Total-P)	0.63	all	<0.001	16949
PTI	Phytoplankton Trophic Index	Eutrophication (Total-P)	0.67 (GAM)	all	<0.001	1500
SPI	Size Phytoplankton Index	Eutrophication (Total-P)	0.30 0.25 0.13	CB N M	<0.0001 <0.0001 <0.05	117 59 29
MFGI	Morpho-Functional Group Index	Eutrophication (Total-P)	0.31 0.07 0.23	CB N M	<0.0001 <0.05 <0.01	117 59 29
J'	Evenness	Eutrophication (Total-P)	0.19 0.07	N CB	<0.001 <0.001	716 559
Cyano bloom intensity	Cyanobacteria biovolume (mg/l)	Eutrophication (Total-P)	0.34 (GAM)	All	<0.001	

Uncertainty and Sampling Guidance

Within-lake variability caused by natural spatial variation, as well as variability related to sampling and analyses was low for phytoplankton (Table 3), although this BQE probably has much higher temporal variability related to sampling frequency (tbc). If excluding temporal variability, the most precise phytoplankton metrics having the lowest within-lake variance are chlorophyll, cyanobacteria biovolume and the taxonomic composition index PTI. The most important variance component for these metrics is sub-sampling. However, as the total within-lake variance is so low for these metrics (ca.5-10%), the error caused by sub-sampling is minor. This may have been relatively low in the WISER field exercise as counters all attended training workshops to standardize counting methods and identification prior to sample analysis.

Table 3. Metric precision given as the proportion of total variance (within- and between- lake variance) due to within-lake variability, and major variance component. See table 2 for explanation of metrics. Data taken from Thackeray et al. (2011).

Metric	Within lake variance (excluding temporal variability*)	Major variance component (excluding temporal variability*)
Chl-a	0.04	Sub-sampling
PTI	0.12	Sub-sampling
SPI	0.35	Analyst
MFGI	0.14	Sub-sampling
J'	0.31	Analyst
Cyanobacteria biovolume	0.06	Sub-sampling

Discussion

Recommendations of metrics for IC and National MS schemes

Annex V of the WFD specifically outlines three features of the phytoplankton quality element that need to be considered in this assessment for lakes (abundance, composition and blooms). The review of national metrics revealed that many MS used chlorophyll a as a biomass or abundance metric and many used some form of index based on indicator taxa lists and their trophic scores as a composition metric (e.g., Brettum 1989, Dokulil 2005, Salmaso 2006). Based on both the strength of the relationships (Table 2) and metric uncertainty (Table 3) our WISER analysis would strongly support this choice of metrics, with chlorophyll and the PTI having both the strongest relationships with TP and also some of the lowest within-lake variance. Our analysis shows that non-taxonomic morpho-functional approaches (SPI & MFGI) had weaker relationships with TP and higher within-lake variance (particularly the SPI). The reasons for this are not clear but could be due to the smaller number of indicator groups, compared with genera- or species-based indeces, and greater weighting to biovolume estimates in the size-based indeces. The uncertainty in the latter could potentially be reduced through improved counter training or more automated methods for assigning size-classes, such as the use of flow cytometry.

Of the two bloom metrics developed and tested in WISER, cyanobacterial biovolume is recommended over evenness as it had a significant relationship with pressure (Table 2) and had very low levels of within-lake variance (Table 3). This metric effectively represents the intensity of summer blooms. What is an acceptable frequency of blooms has not, however, been investigated in WISER. The wording of the normative definition in Annex V of the WFD mentions “persistent blooms during summer” which tends to suggest high frequency monitoring within a summer is needed. Based on labour-intensive in-lake sampling and counting methodologies this is clearly not practical for any Member State. New technologies based on fluorimetry (e.g. BBE algal torch), citizen monitoring of cyanobacterial blooms (e.g. Finland) or new hyper-spectral European satellite platforms (e.g. MERIS and Sentinel 2; Hunter et al., 2011), could, however, make higher frequency monitoring a real possibility in the near future. Despite this, the WISER temporal uncertainty analysis appears to suggest that, for the cyanobacteria biovolume metric, inter-annual variability is greater than the monthly variability within a single summer and, therefore, frequency of sampling may be better targeting different years (see following section).

Recommendations for minimising uncertainty in classification

The phytoplankton community is notoriously dynamic over a year, and even within a season. Developing an ecological assessment scheme, specifically in relation to nutrient pressures, requires minimising the effects of seasonal variability associated with the changing physical and biological structure of the water column and magnifying the signal related to nutrient pressures.

Table 5. Minimum recommended sampling frequencies for three phytoplankton metrics in three GIGs. The number of months and years mean 1 sample taken for each of the number of months in each of the number of years. For example for NGIG, chlorophyll a should be sampled at least once in 2 different months in each of 3 different years or once in 3 different months in each of 2 different years, meaning 6 samples altogether.

	CB-GIG	M-GIG	N-GIG
Chlorophyll *a*	3 months for 4 years	3 months for 3 years	2 months for 3 years or 3 months for 2 years
PTI	2 months for 4 years or 1 month for 6 years	3 months for 3 years or 1 month for 6 years	3 months for 3 years or 1 month for 6 years
Cyanobacteria	1 month for 6 years	1 month for 6 years	1 month for 6 years

The phytoplankton community is, however, notoriously diverse and dynamic. Developing an ecological classification specifically in relation to nutrient pressures requires minimising the effects of seasonal variability associated with the changing physical and biological structure of the water column and magnifying the signal related to nutrient pressures.

Combining metrics for whole BQE assessment

- Examples from IC (N and CB GIGs) and individual MS (Med GIG, Germany)
- Case-studies of where particular metrics, and their combination, affect status assessment (Geoff's UK case-study? Brenda's Irish analysis? Caridad's Med GIG analysis?)
- Pros and cons of different combination rules

Wider conclusions on assessment of eutrophication and recovery

Despite it being widely acknowledged as representing important impacts of eutrophication on lake ecosystems, phytoplankton composition has rarely been adopted as a component of modern lake classification schemes. The requirement of expert skills in identification and the complexity of interpretation limited their routine application (Fozzard et al. 1999). The WFD has changed this. Substantial efforts in collecting consistent phytoplankton data across Europe has allowed robust quantitative relationships to be developed between composition and nutrient pressure. The dynamic nature of phytoplankton communities can be overcome by either frequent monthly sampling where possible (e.g. chlorophyll a) or by restricting the seasonal window that metrics operate in (summer composition and blooms metrics).

However, there are still issues to resolve. The WFD outlines the need for these classification schemes to represent the health of the structure and function of the water body, so metrics need to represent more than just TP, and represent what we believe eutrophication is all about more widely. Metric strength has largely been assessed based on relationships with TP. However, some metrics which show weaker (but still highly significant) relationships with TP may also be of value. For example, the cyanobacterial bloom metric represents the most widely accepted impact of eutrophication on water use for recreation and water supply, and makes WFD targets relevant to ecosystem services valued by the general public. Other metrics such as SPI and MFGI may in fact represent important functional impacts of eutrophication and their usage should be examined further.

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Acknowledgements

This paper is a result of the project WISER (Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery) funded by the European Union under the 7th Framework Programme, Theme 6 (Environment including Climate Change) (contract No. 226273). We would like to thank all the phytoplankton counters and national data providers. These include (figures in brackets give the number of WBs):

Phytoplankton counters (WISER field exercise)

Name	Institute	Country
Aimar Rakko	EMU	Estonia
Kairi Maileht	EMU	Estonia
Marko Jarvinen	SYKE	Finland
Christophe Laplace	CEMAGREF	France
Ute Mischke	IGB	Germany
Annette Tworeck	LBH	Germany
Martina Austoni	CNR	Italy
Panzani Pierisa	CNR	Italy
Virdis Tomasa	University of Sassari	Italy
Maria Antonietta Mariani	University of Sassari	Italy
Birger Skjelbred	NIVA	Norway
Camilla Hagman	NIVA	Norway
Agnieszka Pasztaleniec	IEP	Poland
Malgorzata Poniewozik	IEP	Poland
Caridad de Hoyos	CEDEX	Spain
Ana Negro	University of Salamanca	Spain
Roser Farres	CSIC	Spain
Tatiana Caraballo	CSIC	Spain
Jordi Catalan	CSIC	Spain
Stina Drakare	SLU	Sweden
Sarah Pritchard	Beacon Biological	UK
Laurence Carvalho	CEH	UK
Mitzi De Ville	CEH	UK
Rene Groben	CEH	UK
Bill Brierley	EA	UK
Gina Henderson	ENSIS	UK

Mediterranean GIG (Data manager: Caridad de Hoyos, CEDEX-CEH)

Portugal: Instituto da Água, I.P. (INAG) (18)

Spain: Ministerio de Agricultura, Alimentación y Medio Ambiente (122), Centro de Estudios Hidrográficos (CEDEX-CEH) (46).

France: Institut national de recherche en sciences et rechnologies pour l´environnement et l´agriculture (IRSTEA) (6).

Italy: Università degli Studi di Sassari. Dipartimento di Scienze Botaniche, Ecologiche e Geologiche (DiSBEG) (18).

Cyprus: Ministry of Agriculture, Natural Resources and Environment, Water Development Department (MANRE-WDD) (7).

Greece: Maria Moustaka, Aristotle Unniversity of Thessaloniki (1)

Central-Baltic GIG (Data manager: Ute Mischke, IGB)

Estonia: Estonian University of Life Sciences (EMU) gathered for the state monitoring programme supported by the Estonian Ministry of Environment.

Latvia: Sandra Poikane, Latvian Environment, Geology and Meteorology Centre

Lithuania - EPA Lithuania

Denmark: National Environmental Research Institute, University of Aarhus.

Netherlands: Rijkswaterstaat (RWS)

Belgium: Jeroen Van Wichelen, Ghent University (UGENT)

France: Institut national de recherche en sciences et rechnologies pour l´environnement et l´agriculture (IRSTEA).

Germany: Data from German water bodies were kindly provided by the following institutions of the German Federal States: Landesamt für Umwelt, Gesundheit und Verbraucherschutz Brandenburg (LUGV; 127), Ministerium für Landwirtschaft, Umwelt und Verbraucherschutz Mecklenburg-Vorpommern (MLUV, Seenprogramm, 65), Landesbetrieb für Hochwasserschutz und Wasserwirtschaft Sachsen-Anhalt (LHW, 5), Landesamt für Landwirtschaft, Umwelt und ländliche Räume Schleswig-Holstein (LLUR, 13), Senatsverwaltung für Gesundheit, Soziales und Verbraucherschutz Berlin (SenGUV, 12), Niedersächsische Landesbetrieb für Wasserwirtschaft, Küsten- und Naturschutz (NLWKN, Sulingen, 1).

Poland: Data were provided partly by the Institute of Environmental Protection - National Research Institute. The Inspection for Environmental Protection provided data obtained within the framework of state environmental monitoring

United Kingdom - Scottish Environment Protection Agency (SEPA) and the Environment Agency for England & Wales (EA)

Eastern-Continental GIG (Data manager: Gabor Borics, EPI)

Hungary: Environmental Protection Inspectorate for Trans-Tiszanian Region

Romania: Ministeriul Meduli şi Pădurilor (MMP) (10).

Northern GIG (Data manager: Geoff Phillips, EA)

Finland: Finnish Environment Institute (SYKE)

Sweden: Swedish University of Agricultural Sciences (SLU)

Norway: Norwegian Institute for Water Research (NIVA)

United Kingdom - Scottish Environment Protection Agency (SEPA) and the Environment Agency for England & Wales (EA)

Ireland: Environment Protection Agency (EPA)