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Ecological threshold responses in European lakes and their applicability for the Water Framework Directive (WFD) implementation: synthesis of lakes results from the REBECCA project

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Abstract The objective of this synthesis is to present the key messages and draw the main conclusions from the work on lakes in the REBECCA project, pointing out their links to theoretical ecology and their applicability for the WFD implementation. Type-specific results were obtained from analyses of large pan-European datasets for phytoplankton, macrophytes, macroinvertebrates and fish, and indicators and relationships showing the impact of eutrophication or acidification on these biological elements were constructed. The thresholds identified in many of the response curves are well suited for setting ecological status class boundaries and can be applied in the intercalibration of classification systems. Good indicators for phytoplankton (chrysophytes, cyanobacteria) and macrophytes (isoetids and charaphytes) responses to eutrophication were identified, and the level of eutrophication pressure needed to reach the thresholds for these indicators was quantified. Several existing metrics developed for macrophytes had low comparability and need further harmonisation to be useful for intercalibration of classification systems. For macroinvertebrates, a number of metrics developed for rivers turned out to be less useful to describe lake responses to eutrophication and acidification, whereas other species based indicators were more

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promising. All the biological elements showed different responses in different lake types according to alkalinity and humic substances, and also partly according to depth. Better harmonisation of monitoring methods is needed to achieve better precision in the dose–response curves. Future research should include impacts of hydromorphological pressures and climate change, as well as predictions of timelags involved in responses to reduction of pressures.

Keywords Acidification · Dose–response curves · Ecological status · Eutrophication · Intercalibration · Non-linearity · Water

Introduction

The objective of this synthesis article is to extract the main results presented in the other articles in this special issue, to draw the main scientific conclusions and point out the applicability of these for the Water Framework Directive (WFD) implementation process. The article starts with some introductory parts justifying the need for the knowledge provided in this special issue for the development and intercalibration of WFD-compliant classification systems, and the linkage to ecological theory of threshold responses to perturbations. The main methodology used in the work on lakes in the REBECCA project is summarised before presenting the key messages from all the separate articles. In the final part, the general response patterns found for the different biological elements to the pressures of eutrophication and acidification are compared, focusing on the ecological thresholds detected along the pressure gradients. The applicability of the conclusions for the development and intercalibration of classification systems are highlighted, and the remaining knowledge needs are listed.

Knowledge needs for WFD

The EU Water Framework Directive (WFD) (EC 2000) represents a truly ecological approach to water management in Europe. In order to enable a successful implementation of the Directive, considerable new scientific efforts and knowledge of aquatic ecosystems are needed, in particular to assess the WFD objective of good ecological status. The WFD is the first attempt to move from environmental quality standards (specification of the maximum level of contamination that is legally permissible in a given part of the physical environment) towards ecological quality standards; i.e. a minimum acceptable state of ecosystems and their biological components, with a corresponding legal obligation that no deterioration below that standard should be permissible (Howarth 2006). Hence, ecological quality standards for flora, fauna and habitats are intended to serve as a mandatory baseline to ensure normal structure and function for aquatic ecosystems. The standards must be specified quantitatively in terms of composition, abundance and diversity for each component, and are supported by legal obligations to ensure their realisation. In practice, the Directive requires assessment of the ecological status of water bodies as the basis for river basin management plans, including planning of adequate management measures to achieve the good ecological status target. In the Directive Annex V, this target is defined as slight deviation from type-specific reference conditions for the water body, and should be measured by both biological elements, such as phytoplankton, benthic flora, macroinvertebrates and fish, as well as non-biological supporting elements, i.e. different physico-chemical and hydromorphological elements. The major challenges for the implementation of the WFD are to quantify the reference conditions for major types of water bodies, and to set boundaries for all the ecological status classes: high, good, moderate, poor and bad. These boundaries must be based on the normative definitions for the different elements as given in Annex V of the WFD, translated into quantitative measurable indicators for these elements and also related to different pressures, in order to be operational for water management.

At the start of the REBECCA project in 2004, only limited knowledge existed on indicators and indicator–pressure relationships for different types of European lakes. These indicators were mainly based on results from previous EU projects, such as ECOFRAME, dealing with very shallow lakes (Moss et al. 2003). Thus, the major objective for the REBECCA studies on lakes has been to develop such indicators and construct dose–response relationships for a variety of lake types showing the ecological impact on phytoplankton, benthic flora, macroinvertebrates and fish of important human pressures, such as eutrophication and acidification (see also Preface to this issue).

Scientific support for the development and intercalibration of WFD classification systems

The WFD requires each member state to develop and intercalibrate WFD compliant classification systems for assessing ecological status of water bodies. Before the REBECCA project, this work was initiated by most EU member states, but none of them had completed their classification systems, partly due to lack of good indicators for several biological elements and partly due to lack of knowledge on how these indicators will respond to different pressures. The intercalibration of these classification systems, therefore, was slower and less conclusive than expected (see EU Intercalibration report 2007), and the need for input from scientific projects like REBECCA was urgent and strong. This concerns both the assessment of reference conditions for most biological elements in all common lake types, as well as the response curves for the different elements to different pressures.

The intercalibration of WFD-compliant classification systems for lakes was organised by the ECOSTAT working group under the common WFD implementation strategy (CIS) agreed by the EU commission, member states and Norway. For the purpose of intercalibration of classification systems, the different European countries that have common, comparable types of water bodies were assembled into 5 geographical (regional) intercalibration groups (GIGs): Northern, Central/Baltic, Atlantic, Alpine and Mediterranean (EU Intercalibration guidance). In order to support the intercalibration work within these GIGs, the partners of the REBECCA studies on lakes compiled large pan-European datasets (see Materials and methods below), and used these to analyse type-specific dose–response curves for all regions and types where data were sufficient for analyses. Many of the response curves derived from these datasets were found to be non-linear and exhibited thresholds relevant for setting the WFD good/moderate status boundary. Such thresholds are especially useful if they allow discriminating stable from unstable and/or desirable from undesirable communities, in accordance with the WFD normative definitions (WFD Annex V). The results thus also contribute to building of ecological theory on thresholds or tipping points of ecosystems (see below).

Linkage to ecological theory: threshold responses

Ecosystems often show non-linear responses to large perturbations (Millenium Ecosystem Assessment 2005). Although such responses may be smooth unimodal or bimodal functions, many typically non-linear responses are S-shaped, exhibiting clear thresholds or tipping points that is caused by ecological regime shifts. These responses have become established as a framework in ecological theory for understanding of non-linear dynamics (Scheffer and Carpenter 2003). A threshold or change point is defined as an abrupt change with respect to an environmental factor or stressor, which strongly modifies a defined system or community. Though the existence of thresholds and discontinuities in responses of communities to perturbations had already been demonstrated by modellers in the seventies (May 1977), the 'threshold' concept was not explicitly represented in the ecological literature before early 2000. Especially the observations of the switching between two stable states in shallow lakes (Scheffer et al. 2001; Scheffer and Carpenter 2003) enhanced further research on non-linear dose–response relationships.

Change points can be related to hysteresis or resilience, i.e. when an ecosystem has some inherent stability that keeps it from changing much in response to a change in pressure or perturbation until the threshold is reached. Beyond the threshold, the stability breaks down and large changes occur, either expressed as large fluctuations in an unstable system (Lyche 1990, Andersen 1997) or eventually an abrupt shift to a quite different (and often undesirable) stable state (Scheffer et al. 2001). Moreover, the decreased stability results in biodiversity decrease and loss of certain interactions, as reviewed by McCann (2000) and further studied by Rooney et al. (2006). For example, nutrient loading to aquatic ecosystems may result in the dominance of certain components and energy flows, reducing the originally diverse food web structure and functioning.

For some relationships, nonlinear responses are very obvious, as they are defined by the level of a parameter, beyond which it has deleterious effects on the biota. For example, toxic effects of low pH on fish occur in a rather short range across the possible pH range found in lakes and rivers, while little response is observed above this threshold (see Hesthagen et al.

2008, this issue). The same applies for many toxic pollutants. In order to identify levels of critical nutrient loads in aquatic ecosystems, research originally focused on thresholds of internal nutrient loading in stratified lakes (Nürnberg 1984). Scheffer et al. (2001) defined the threshold for very shallow lakes as a critical level of phytoplankton abundance above which macrophyte cover and zooplankton abundance decrease drastically. In one state (A), the system is characterised by low phytoplankton abundance, rich cover of submerged macrophytes and high zooplankton biomass. The zooplankton escapes fish predation due to the presence of macrophytes, and keeps the phytoplankton at low numbers. The growth of macrophytes keeps dissolved nutrients levels constantly low. If one of the stabilizing mechanisms breaks down, such as the decrease of zooplankton grazers and/or strong light limitation of macrophytes caused by abundant phytoplankton, the system flips into another state (B), which is characterised by high phytoplankton abundance, the absence of macrophytes, reduction of zooplankton due to predation by fish and little grazing control by zooplankton on phytoplankton. The combination of these food-web interactions stabilizes the system on either side of the threshold (so-called feed-back loops).

Algal biomass increases approximately linearly with nutrient enrichment (Phillips et al. 2008, this issue and refs. therein). However, the effect of the increased algal biomass on light extinction at a given depth is exponential, meaning that linear changes in chlorophyll-a have exponential effects on underwater light-climate. Thus, linear changes in phosphorus and chlorophyll will have highly non-linear effects on macrophytes in lakes deeper than their natural colonisation depth.

Distinguishing thresholds in a response curve in an objective way is a big challenge, since subjective visual inspection can give a different result than an objective statistical test. In this special issue this has been clearly demonstrated in the paper on macrophytes by Penning et al. (2008a, this issue), in which the visually based threshold for sensitive isoetids against total phosphorus (TP) is different from that indicated by a quantile logistic regression analyses. This does not necessarily mean that the visual inspection is less valid, since statistical tests are much more affected by noise in the dataset. Visual inspection, on the other hand, is especially problematic when the distribution of points is very uneven with numerous points lying on top of each other, and thus being invisible. The optimal method to identify a threshold therefore depends upon the type of trend detected, as well as on the quality and quantity of the dataset. An alternative to regression analysis for detection of thresholds is probabilistic analyses, where e.g. the quantile distribution of a parameter in two or more groups of sites are compared (e.g. probability to surpass a critical indicator level in reference versus impacted sites).

Materials and methods

Compilation of existing data from lakes in Europe

The WFD has defined a set of lake types (WFD Annex II, System A) for each ecoregion of Europe (later merged into Geographical Intercalibration Groups, GIGs), although also alternative lake types can be defined (WFD Annex II, System B). Lakes within each type are expected to have similar reference conditions and similar ecological responses to pressures. An important task for the REBECCA work on lakes was to analyse type-specific relationships. We, therefore, compiled data from several countries for each biological quality element (BQE; phytoplankton, macrophytes, macroinvertebrates and fish), as well as abiotic data from the same sites, including typology variables, such as alkalinity and watercolour (humic content) and physico-chemical pressure data, such as total phosphorus (TP) or chlorophyll for eutrophication, and pH or ANC for acidification. We could thus obtain more observations per lake type, and provide more precise type-specific relationships and reference conditions than if we had used national datasets only. Moreover, combining data from several countries enabled us to cover a larger part of the pressure gradient (such as TP or pH), thus allowing analyses of biological responses along most of the pressure gradient.

An overview of the database contents is given in Table 1 (see also Moe et al. 2008, this issue). The data, except those for fish, were organised as multi-table, relational databases in Microsoft Access for each BQE, including separate tables for information on stations, chemical samples, chemical values, biological samples, biological values, and taxonomy. Such a structure allowed aggregation and combination of the data in various ways needed for exploratory

Table 1 Overview of the REBECCA Lakes database contents

Region	Countries	Chlorophyll *a* (80,978 samples)		Phytoplankton (8,726 samples)		Macrophytes (16,022 samples)		Macroinvertebrates (3,801 samples)		Fish (5,248 samples)	
		No. of lakes	No. of ref. lakes	No. of lakes	No. of ref. lakes	No. of lakes	No. of ref. lakes	No. of lakes	No. of ref. lakes	No. of lakes	No. of ref. lakes
Alpine	AT, DE, IT,	66	11	10	1	0	0	13	3	0	0
Atlantic	IE, UK	27	7	66	14	123	40	0	0	0	0
Central/Baltic	BE, DE, DK, EE, FR, HU, LT, LV, NL, PL, RO, UK,	848	38	503	33	226	66	0	0	79	0
Mediterranean	CY, ES, IT, RO	132	1	80	7	19	8	18	0	6	0
Northern	FI, IE, NO, SE, UK	4419	483	791	413	1074	423	1135	471	2219	0
Sum	20 countries	5,492	1,664	1,450	468	1,442	537	1,166	474	2,304	0

Information including reference status and typology factors is available for most of the stations. Most of the biology samples can be linked with chemistry samples (such as TP or pH)

analyses (e.g. aggregation of different time periods, or different taxonomic levels).

The amount of data received was much higher than expected, and included data from several collaborators outside of the REBECCA consortium. Despite a great effort at standardising units and taxonomic names, still much heterogeneity remained in the data that could not be reduced, e.g. because sampling methodology differed among different countries. These are important sources of uncertainty that made it more challenging to interpret the results of the multi-national analyses.

Methods and models used for the data analyses

Ecological data pose several methodological challenges, such as: (1) non-linear responses with abrupt changes and thresholds; (2) complex community responses; (3) high uncertainty associated with the data. The main statistical methods used for data analyses in REBECCA have been selected to deal with these challenges. They are presented below (see also in Moe et al. 2007).

Exploring non-linear relationships: non-linear regression methods

Many of the responses analysed in this project were non-linear, exhibiting threshold-like behaviour. Thus non-linear regression methods were required. We have applied a set of different non-linear regression models, including GAM (generalised additive models) and quantile regression. The so-called non-parametric regression models fit a regression curve as a large number of segments with smoothed joints along the x-axis. Thus, it is possible to explore the structure of the relationships, and to discover non-linearities and thresholds, in a more flexible way than for ordinary parametric regression models. This combination of approaches allows exploring different properties of the relationships between pressure and response.

Non-parametric regression models, generally require large datasets; a requirement that has been possible for meet with the large databases compiled, including data from 20 countries for most of the biological elements (phytoplankton, macrophytes, macroinvertebrates and fish). Many articles presented in this special issue are based on non-linear regression models (Ptacnik et al 2008; Penning et al. 2008a;

Schartau et al 2008; Hesthagen et al. 2008). Examples of different types of regression analyses are given in Fig. 1.

Exploring community structures: multivariate methods

Multivariate analyses can be used for analysing the species/samples abundance (or biomass) matrices. These are often used in biological monitoring of environmental impact and in more fundamental studies in community ecology, together with associated physico-chemical data. The methods normally make few, if any, assumptions about the form of the data. 'Non-metric' ordination and permutation tests are fundamental to this approach.

Most calculation methods are rather robust and thus widely applicable, leading to greater confidence in interpretation of community patterns. The methods have been adopted worldwide, particularly in marine studies, but also increasingly in terrestrial, freshwater and palaeontology works.

There are numerous multivariate assessment methods available, all having the following underlying assumption: based on a dataset, clusters can be found by comparing the contents of the individual samples. Samples that are similar to each other are grouped together, using matrix similarity assessment methods.

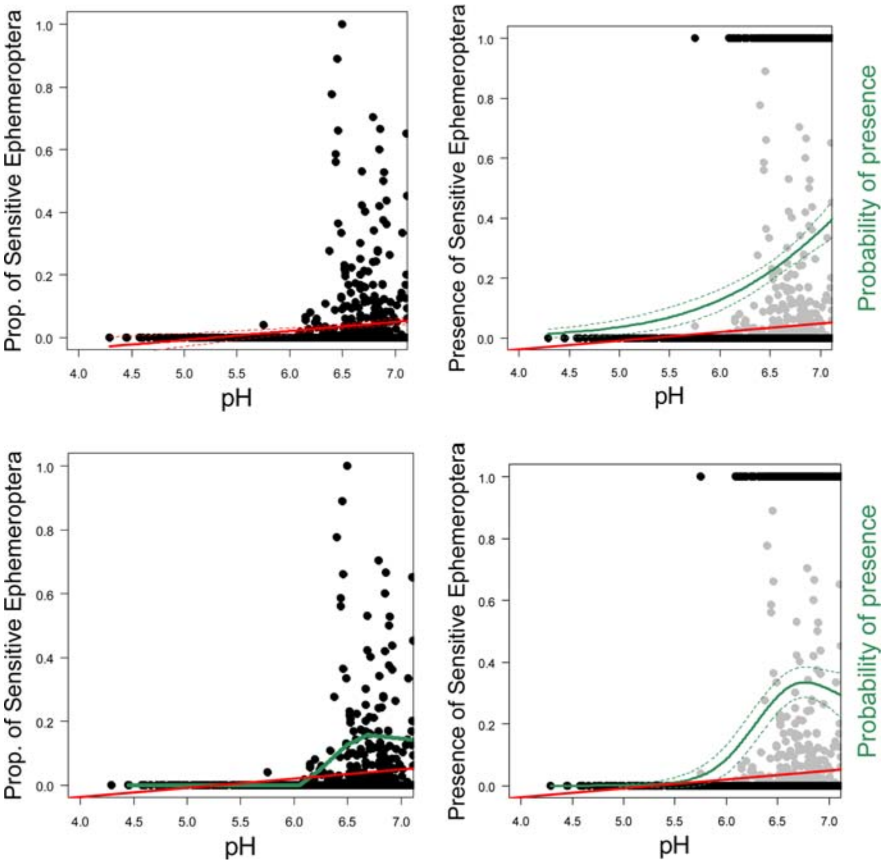

Fig. 1 Different types of univariate regression models applied to the biological metric Proportion of Sensitive Ephemeroptera, as an indicator of acidification (pH). Upper left: ordinary linear regression (also shown in the other plots for comparison). This method clearly gives a poor fit to the data. Upper right: logistic regression. The method transforms abundance to presence/absence, and thereby loses much information, but seems to fit the data better. However, it does not capture the threshold indicated by the data. Lower right: logistic regression, non-parametric version. This method also displays a threshold. Lower left: 90% quantile regression (non-parametric). This method also shows a threshold, and also uses the abundance information in the data. Dashed curves show 2× standard errors

This allows identification of specific groups within biological community data on various levels of detail. Often there is a suite of environmental (physico-chemical) data available for each biological data point. The two datasets together (physico-chemical and biological) give the user a large amount of information on the functioning of the studied system. Below is an example of a CCA analysis (Canonical correspondence analysis) on phytoplankton data (Fig. 2). Such analyses were often used in the initial phases of data analyses to identify groups of indicator taxa showing approximately similar environmental preferences.

Exploring uncertainties: Bayesian methods

Probabilistic methods are being used increasingly for ecological modelling and analysis. Bayesian models are based on and predict probability distributions, and incorporate uncertainties in a more explicit way than the more common classical or "frequentist" statistics do. In the REBECCA work on lakes, two different Bayesian approaches have been applied for two particular purposes:

(1) Estimation of target concentration of TP for obtaining good ecological status for lakes, according to proposed boundary values for chlorophyll a. A hierarchical Bayesian regression model was used for this purpose.

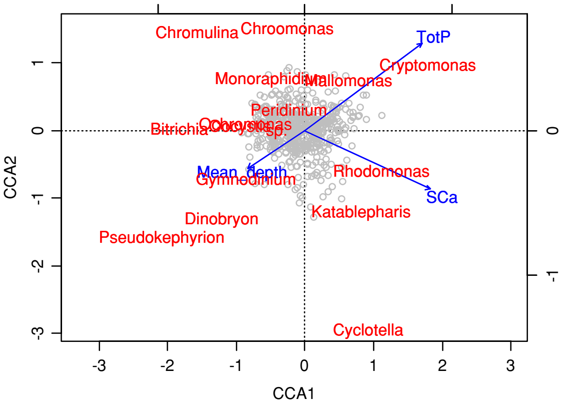

Fig. 2 Example of a multivariate analysis (Canonical correspondence analysis) of phytoplankton data, linking the relative abundance of different phytoplankton genera to chemical and physical variables. The position of the genera shows their optimum occurrence relative to gradients of calcium estimated from survey data (SCa), total Phosphorus (TP) and mean depth, whereas the grey dots are the single samples used for the analyses

(2) Classification of single lake status according to the WFD, using information from four different biological elements and taking into account the uncertainty associated with each element. A Bayesian Network approach was used for this purpose. These analyses are published in the REBECCA report D12 (Moe et al. 2007).

Main results

The REBECCA project work on lakes has been to develop indicators and analyse relationships for four groups of organisms (i.e. biological elements *sensu* WFD): phytoplankton, macrophytes, macroinvertebrates and fish. The impact of eutrophication pressure has been assessed for the first three elements, whereas acidification impact has been assessed for the macroinvertebrates and fish. The detailed results from development and testing of ecological indicators for the main groups of organisms in lakes and their relationships with the above mentioned pressures (eutrophication and acidification) are reported in different articles in this special issue (see also further details in Lyche Solheim et al. 2006). This synthesis article is based on the major key messages and highlights from all the articles.

Chlorophyll reference conditions

Phytoplankton chlorophyll is widely used as a measure of phytoplankton biomass, and thus was selected early on in the intercalibration process as a key indicator of the ecological impact of eutrophication in lakes. The WFD requirement to define lake type-specific reference conditions required an analysis of the factors shaping chlorophyll concentrations in the absence of nutrient pressures. Lake data from more than 500 European reference lakes was collated from national datasets from individual Member States through partners in the REBECCA Project and from the GIG coordinators. Reference lakes were identified using consistent pressure criteria (see Carvalho et al. 2008, this issue).

For each reference lake, data were collated on chlorophyll concentration, altitude, surface area, mean depth, alkalinity, humic content and GIG region. In order to derive type-specific reference chlorophyll concentrations, the median statistic of a population of lakes of the same type was considered appropriate. Higher percentile statistics, such as the 75th and 90th percentiles, were also summarised as a suitable measure for defining the high/good status class boundary, as this would mean that, appropriately, a high proportion of reference lakes would be classified as high status.

Reference chlorophyll concentrations varied with lake type. The median values generally ranged between 2.0 and 7.0 μg l^{-1} with lowest values for deep, low alkalinity and clear water types and highest values for very shallow, high alkalinity and humic lake types (Carvalho et al. 2008, this issue—Table 4). Analysis of the dataset, however, revealed significant gradients in response. For example, deep lakes had relatively lower chlorophyll concentrations and were less variable, whereas very shallow lakes had higher concentrations and were also more variable (Fig. 3).

For this reason, multiple regression models were also established for predicting site-specific chlorophyll reference conditions for individual lakes, based on their typology data (humic type, mean depth and alkalinity). These models are particularly applicable for lakes that do not fall within an intercalibration type, and this approach also reduces error in chlorophyll reference conditions for lakes that lie close to a type boundary.

Chlorophyll/phosphorus relationships

Reliable models to link nutrient and chlorophyll a concentrations are important for managers to plan appropriate nutrient reduction measures needed to achieve or prevent exceedance of the intercalibrated chlorophyll boundaries for good ecological status. We used simple linear models to explore lake type and regional differences in the relationships of chlorophyll *a* with total phosphorus (TP) and total nitrogen (TN) (Phillips et al. 2008, this issue). In all the cases, we found TP to be a better predictor of chlorophyll than TN, confirming the widely accepted view that phosphorus is usually the limiting nutrient for phytoplankton in lakes (Schindler 1977), although late-summer depletion of N and N-limitation is quite common in eutrophied lakes (Vollenweider 1975). We found no significant regional differences, but marked differences in response for some lake types. Deep lakes produced lower yields of chlorophyll per unit phosphorus than the shallow and very shallow lakes. This fits ecological theory, with light limiting production more in deep lakes where the mixing depth is often larger than the euphotic depth. In more shallow lake systems light becomes limiting for primary production only in highly eutrophic or turbid lakes, whereas less eutrophic and less turbid shallow lakes are less prone to be light limited. The higher chlorophyll per unit of TP found in shallow lakes than in deep lakes is also most likely related to a more rapid recycling of nutrients, maintaining production.

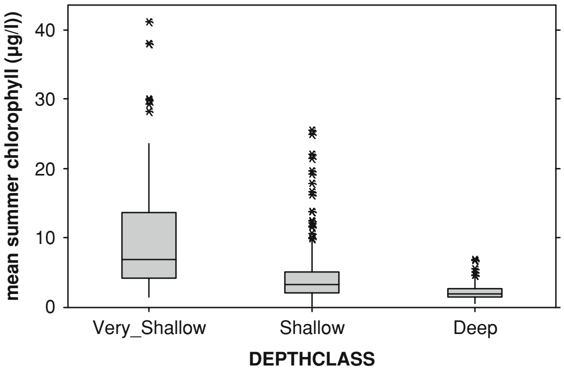

Fig. 3 Boxplots comparing chlorophyll concentrations in reference lakes for three depth types. The middle line indicates the median value, the top and bottom of the box are the third (75%) and first quartile (25%) respectively and the top and bottom of the vertical lines are the 10th and 90th percentiles of the data distribution. The asterisks represent extreme outliers

In contrast to other studies (Edmundson and Carlson, 1998; Havens, 2003) we found no differences between the phosphorus–chlorophyll relationship in humic and clear water lakes. The humic lakes had higher phosphorus concentrations, but also higher chlorophyll and no evidence of lower chlorophyll yields per unit phosphorus. We found the least significant relationships in high alkalinity shallow and very shallow lakes. In several lakes of these types high total phosphorus did not give correspondingly high chlorophyll. This is not unexpected given the importance of macrophytes, as well as top-down control by zooplankton grazing in these lakes (Scheffer, 1990). Moreover, a large part of TP may also be unavailable Ca-bound P resuspended from bottom sediments. Some of these lakes may have high turbidity even in reference conditions.

These variations in response highlight the need for water managers to use models carefully when establishing target concentrations for nutrients. The use of such type-specific models developed from a population of European lakes should provide better predictions for chlorophyll than other widely used relationships that do not take differences between lake types into account, and that may not even be representative for all lake types (OECD 1982). However, they can never predict precisely the outcome for a particular lake (Reynolds 1980). Therefore, we have provided error boundaries for our models to facilitate their use by managers (see Table 3 in Phillips et al. 2008, this issue). Thus target phosphorus concentrations can be identified below which 50% of the lakes (mean regression line) or 90% of the lakes (upper confidence limit of the mean regression line) would have the desired chlorophyll concentration, the latter requiring a more stringent phosphorus target. The choice of phosphorus target needs to be based on a certain selected degree of certainty that the desired chlorophyll level will be achieved. The selection of the degree of certainty is commonly a trade-off between risk and potential costs.

Phytoplankton responses to eutrophication

The response patterns for various classes of phytoplankton were analysed using a large dataset from Northern lakes (NO, SE, FI; UK) and a non-linear regression technique (see Ptacnik et al. 2008, this issue). These functions allowed estimation of confidence intervals to estimate the uncertainty around the main trend line. The identified response curves were generally well in agreement with ecological theory and earlier empirical data regarding the location of optimum occurrence for different phytoplankton classes along the pressure gradient: the relative abundance of chrysophytes decreased with increasing eutrophication, the pennate diatoms showed an optimum in mesotrophic conditions, whereas the Cyanobacteria increased abruptly above a certain type specific threshold of chlorophyll. The most abrupt threshold was found in low alkalinity lakes, in which Cyanobacteria have insignificant abundances below 5 μg chlorophyll-a l^{-1}, but often very high abundances above this threshold. Clear differences in response were found between humic lakes and clear-water lakes.

Macrophyte community changes due to eutrophication pressures

Macrophyte species were characterised as sensitive, tolerant or indifferent to eutrophication pressure based on analyses of macrophyte data from more than 1,000 lakes from 12 countries. The results depend on the dataset used and the homogeneity of the monitoring methods applied to obtain the data, but also by the method of identifying indicator taxa. In a comparison of two methods measuring species response to a trophic gradient represented by total phosphorus (%ile occurrence and trophic ranking using CCA), only 48 out of 114 species were characterised similarly with both methods (Penning et al. 2008a, this issue). Therefore, a comparison of data and classification results from different countries, as requested for the intercalibration of classification systems by the WFD, is hampered not only by the method of data collection, but also by the assessment methods.

Three other indices expressing the status of the overall macrophyte communities in lakes along a eutrophication gradient (using TP as a pressure parameter) were tested: a simple species richness, a trophic index and a lake trophic rank index. Although the trophic index worked well with a national dataset from Norway, the large variability of the general response of the indices to eutrophication reduces their applicability in daily water management. When tested on individual lakes, the response of this index does not necessarily reflect the changes in the lake over time (Penning et al. 2008b, this issue).

The large isoetids, such as *Isoetes lacustris* and *Lobelia dortmanna* for low-moderate alkalinity lakes, and many of the charaphytes, for high alkalinity lakes, seem to be good indicators for reference conditions and good ecological status. Both the assessments based on data (Penning et al. 2008, this issue; Lyche Solheim 2006; Willby et al. 2006) and general expert judgement/literature (Schaumburg 2004; Hill et al. 2000) suggest that isoetids and charaphytes are highly sensitive to an increase in eutrophication pressure (e.g. Blindow 1992, Van den Berg et al. 1999). A clear decline in abundance of large isoetids is noticeable above a specific threshold concentration of TP (ca. 20 μg l^{-1}) (Penning et al. 2008a, this issue).

Results from initial intercalibration of classification systems for macrophytes

The study presented by Toth et al. (2008, this issue) aimed to assess the comparability of classification systems for Central European lakes based on macrophyte indicators for ecological status used in different

Central European and Baltic countries. Classification systems were compared for each of the three major lake types identified: shallow (LCB1) and very shallow lakes (LCB2) with high alkalinity and shallow lakes with moderate alkalinity (LCB3). Cluster and Non-Metric Multidimensional Analyses were applied on a common macrophyte dataset (species composition and relative abundances) collected from 316 lakes sites mainly between 1988 and 2004 within the Central/Baltic region. Based on 27 different national indicator lists from different member states we identified the species occurring in the upper and lower 25th percentiles of the TP gradient to produce a common list of macrophyte indicator species for eutrophication, including indicator species for undisturbed (reference) and disturbed (impacted) environmental conditions. Species with either low or non-agreed indicator value were omitted. Cumulative abundances of the reference and impact groups of indicator species were then tested across the phosphorus gradient of the 316 lake sites. The reference species were those that mainly occurred at the lower end of the phosphorus gradient (TP $< 40\ \mu g\ l^{-1}$), whereas the impact (or tolerant) species mainly occured in the higher end (TP $> 50\ \mu g\ l^{-1}$).

The deviation among the assessment results of the different national methods was quantified using the common macrophyte dataset and indicator list. The multidimensional statistical techniques showed that different types of lakes within the same country had higher similarity than lake sites of the same type from different countries. This finding appears to be caused by regional climate differences, as well as differences in the sampling period and sampling methods. The deviation between assessments results obtained by the various national methods was conspicuous for several lake sites ($1-2$ ecological status classes). The different methods resulted in the same ecological status class for less then one-third of the lakes included in the common dataset. Further harmonisation of macrophyte assessment methods is necessary to obtain better agreement between the various methods.

Macroinvertebrates indicators and responses to eutrophication

A large dataset from 912 lakes mainly from Sweden (63%), Finland (14%) and Ireland (20%) was compiled to analyse the relationship between eutrophication, expressed as total phosphorus (TP) and chlorophyll *a*, and signals (metrics) of environmental quality provided by littoral and profundal invertebrate communities (O'Toole et al. 2008, this issue).

TP optima for most taxa were found to occur in the mesotrophic range (according to the OECD scheme 1982). Species tolerant to eutrophic conditions were the chironomid larvae (*Chironomus plumosus* and *Cryptochironomus defectus*); and two species of tubificids (*Psammoryctides barbatus* and *Potamothrix hammoniensis*), confirming previously published results. However, for the profundal fauna in general, less than half (40%) of the taxa were found to have optima of TP that matched the trophic category stated in the literature.

Macroinvertebrate metrics developed for rivers (Wright 2000) were tested for applicability in the littoral zone of lakes. Although some of them, such as ASPT, BMWP, EPT taxa abundance, % gatherers and collectors and number of families, were significantly correlated with TP or chlorophyll for some lake types, the proportion of variance accounted for by these relationships was low, accounting in all cases for less than 33%, and in most cases for less than 10%. Metric applicability varied across alkalinity bands, which supports the use of alkalinity in the typing of water bodies.

Since the regressions generally explained only a low percentage of variance, the simple metrics developed for rivers seem to be of limited value to assess the impact of eutrophication on littoral macroinvertebrates in lakes. The dataset was however quite "noisy", reflecting a large variety of habitats, variable methods, low seasonal resolution and dominance of samples from oligotrophic waters. Despite such disparate data inputs, we still were able to find some significant relationships showing trends of changes of community structure with increasing nutrient pressure. Further testing of the importance of spatial and temporal variation is needed for the development of sampling strategies, and for the improvements of the precision of responses relating lake macroinvertebrate community changes to eutrophication pressure.

Macroinvertebrate indicators and responses to acidification

Although the acid sensitivity of many invertebrate species from lakes is well known, methods for assessment of lake acidification based on macroinvertebrate samples are less developed than for rivers. The paper by Schartau et al. 2008 therefore evaluated the applicability of existing metrics developed for assessment of river acidification to lakes. Further lake-specific indicators of acidification were also developed and tested. In the latter case, species were characterised and grouped according to their sensitivity to acidification by examining the literature.

All biotic indicators showed significant response in humic lakes, whereas two of them, i.e. the proportion of Ephemeroptera and MILA index did not show any clear relationship with decreasing pH for clear-water lakes (Table 3, Schartau et al. 2008). The following indicators appear most promising for classification of ecological status:

- Both for clear and humic lakes: AWIC F index, and Medin index;
- For clear lakes only: No. of Gastropoda families, proportions of highly tolerant Ephemeroptera and highly sensitive non-EPT taxa;
- For humic lakes only: No. of Ephemeroptera families, proportions of sensitive Ephemeroptera and sensitive EPT taxa.

Most indicators showed a more complex response pattern for humic lakes than for clear lakes. Acid-sensitive indicators often showed a threshold at pH between 5.8 and 6.5. This type of response was more typical for the new lake-specific and species based indicators than for the existing river-based metrics.

Humic content had a positive effect for most acid-sensitive indicators analysed. This supports the hypothesis that humus buffers macroinvertebrates against the detrimental effects of acidity (Hageby and Petersen 1988; Gensemer and Playle 1999). However, in our study we are not able to separate the anthropogenic contribution to low pH values from the contribution by natural acidity in humic lakes. Further work on lake macroinvertebrates should look at the validity of using acid neutralizing capacity (ANC) in combination with dissolved organic carbon (DOC) (cf Lydersen et al. 2004), as a measure of anthropogenically induced acidification (see also Hesthagen et al. 2008).

The strong relationships found between macroinvertebrate communities in the littoral zone of lakes and acidification shows that there is a great potential for further development of assessment systems for ecological quality of lakes based on these indicators. This could be done by combining those macroinvertebrate metrics that show the strongest response to acidification into a new multimetric index.

Fish responses to acidification

Acidification with high concentration of H^+ and elevated levels of inorganic monomeric Al impoverishes fish communities (Driscoll et al. 1980; Schindler et al. 1985; 1991; Rosseland and Staurnes 1994). However, acid neutralizing capacity (ANC) estimated by means of the charge-balanced method is usually included as a potential predictive variable in models for evaluating the biological effects of acidification, rather than pH or inorganic Al (cf. Driscoll et al. 1991). The lower ANC threshold needed to avoid damaged brown trout (*Salmo trutta*) populations in acidified Norwegian lakes has earlier been estimated to be 20 μeq l^{-1} [ANC_{limit}], based on data from a regional study in 1986 (Lien et al. 1996). It has now been suggested to modify ANC (ANC_{mod}) where the permanent anionic charge of organic acids is assumed to be a part of the strong acid anions (Lydersen et al. 2004). They assumed that this may be of particular importance for fish status in humic waters with high concentrations of organic carbon (TOC), because about ⅓ of the dissolved organic matter may be regarded as strong acids.

Hesthagen et al. (2008) estimated ANC both traditionally (ANC_{trad}) and made a modification (ANC_{mod}) as suggested by Lydersen et al. (2004), based on data from 1995. They grouped the lakes into three TOC classes: <2 mg C l^{-1}, 2–5 mg C l^{-1} and $>$ 5 mg C l^{-1} to test the impact of organic (humic) matter on fish survival. Brown trout populations were categorised as unaffected, damaged or extinct on the basis of questionnaires.

Brown trout status was highly related to both pH, inorganic Al and ANC, explaining 45–55% of their variation. A 90% probability of extinction was found at inorganic Al concentrations of 95 μg l^{-1}, and only small damage can be expected at values $<$ 15 μg l^{-1}. A 90 % probability of extinction is expected at pH values of $\leq$ 4.5, whereas no or only small damage can be expected at pH values $>$ 6.5. Fish status was highly related to the humic content of the lakes, as ANC_{limit} was estimated at 33, 73 and $>$ 100 μeq l^{-1} for the three TOC classes, respectively. These differences are due to lower pH and more inorganic Al in humic lakes than in clear water lakes at the same value of ANC. In 1995, the threshold values to avoid fish damage on basis of $\mathrm{ANC}_{\mathrm{trad}}$ and $\mathrm{ANC}_{\mathrm{mod}}$ were 67 and 48 μeq l^{-1}, respectively, compared with 20 and 8 μeq l^{-1}, respectively, in 1986 (cf. Lien et al. 1996; Lydersen et al. 2004). A higher $\mathrm{ANC}_{\mathrm{limit}}$ in 1995 was probably needed because pH was lower and inorganic Al higher at a given ANC than in the mid 1980s. These changes in water chemistry is probably linked to a higher content of TOC in surface waters in recent years, which is evident both from Europe and North America (Skjelkvåle et al. 2001, 2005; Evans et al. 2005).

Fish in humic waters can tolerate higher acidity (lower pH) and more inorganic Al than fish in clear waters. However, as ANC is usually included as a potential predictive variable for evaluating the biological effects of acidification, it is important to take into consideration that the correlation between ANC and pH is dependent on the TOC level, as acidified humic lakes have lower pH and more inorganic Al than clear water lakes at the same value of ANC. A similar relationship has also been suggested in previous studies (Lydersen et al. 2004; Laudon et al. 2005; Teien et al. 2007).

Synthesis and conclusions

Common characteristics of response patterns and links to theoretical ecology

The overall results of these analyses show that various species and groups of species respond nonlinearly to an increase in eutrophication or acidification pressure. Not all species have a clear response to a single pressure, but occur over a wider range of the pressure gradient. These species are generalists and are therefore not well suited as indicator taxa. Thus, caution must be applied when selecting good indicator taxa, and also when interpreting response curves resulting from GAM-analyses, since zero-observations may be caused by other factors than the actual pressure studied (e.g. other pressures, sampling error, unsuitable habitats etc.).

Eutrophication—Species that are sensitive, indifferent and tolerant to eutrophication were identified for phytoplankton, macrophytes and macroinvertebrates. Both sensitive and tolerant taxa were used as indicators, and response-curves along the eutrophication gradient were used to recommend good/moderate class boundaries. For phytoplankton, proportions of Cyanobacteria increased in a threshold-like manner with eutrophication above certain critical chlorophyll-levels, corresponding to certain TP levels. For macrophytes, the isoetides showed a threshold in the opposite direction, i.e. a sudden drop above critical TP levels. Light is probably the crucial factor determining these threshold responses in both phytoplankton and macrophytes (Penning et al. 2008a, this issue, Ptacnik et al. 2008, this issue). The actual threshold levels are however very different: The Cyanobacteria threshold of 6–9 μg chlorophyll l^{-1} corresponding to roughly 12–20 μg TP l^{-1} (based on CHL:TP equations in Phillips et al. 2008, this issue) is lower than the sensitive macrophytes' threshold, which is found at ca. 20–40 μg TP l^{-1}. Thus, the TP concentration and the phytoplankton biomass can increase to twice as high as the threshold for Cyanobacteria dominance before the macrophytes are severely affected.

Thus, phytoplankton seems to be more sensitive to eutrophication pressure than macrophytes. These different sensitivities may be related to the different habitats used by the two groups and the light conditions prevailing in those two habitats: the littoral zone for macrophytes and the pelagic mixed water column for phytoplankton. More light is normally available in the littoral zone than in the pelagic mixed water column, at least in lakes with mixing depth larger than the depth of the littoral zone. Thus, sensitive phytoplankton taxa are most likely to become light limited at a lower P-level than macrophytes, thereby providing competitive advantage for the low light adapted Cyanobacteria.

In this context, the CHL:TP relationships should also be mentioned. A linear relationship can be assumed here, as long as light or other factors than phosphorus are not limiting. Phillips et al. 2008 (this issue) found that the linearity breaks down at TP levels above 100 μg l^{-1}, corresponding to a median chlorophyll concentration of ca. 50 μg l^{-1}. This seems to correspond to a critical level of algal biomass, when self-shading or other limiting factors becomes so strong, that further phosphorus increase has less effect on algal biomass. When taking measures to reduce phosphorus in very eutrophied lakes, little effect can therefore be expected until the phosphorus concentration has decreased below this threshold (100 μg l^{-1}).

Acidification—Responses of macroinvertebrates and fish were analysed as functions of pH or acid neutralization capacity (ANC). Acid-sensitive macroinvertebrate indicators often showed a threshold at pH between 5.8 and 6.5 (Schartau et al. 2008), with a positive effect of humic matter for most of the acid-sensitive indicators. Fish in humic waters can also tolerate higher acidity (lower pH) than fish in clear waters, similarly to invertebrates, mostly due to lower toxicity of Al in humic waters. However, as ANC is usually included as a potential predictive variable for evaluating the biological effects of acidification, it is important to take into consideration that the correlation between ANC and pH is dependent on the TOC level, as acidified humic lakes have lower pH and more Al than clear water lakes at the same value of ANC. The increase of humic acids in Northern lakes during the last decades most likely is responsible for the increased threshold of ANC found for brown trout in 1995 compared to 1986, as well as for the increased threshold of ANC found for humic lakes versus clear-water lakes (Hesthagen et al. 2008).

Conclusions—Threshold responses like those identified in many of the papers in this issue show a sudden shift of species composition, which can be linked to the breakdown of ecological stability of the natural biological communities. If pressure exceeds the threshold value, the sensitive species disappear rapidly, and there is an abrupt transition to highly unstable, fluctuating communities or to a different, often undesirable, stable state dominated by tolerant taxa. These thresholds are therefore crucial to identify for use in lake management, as they are critical boundaries not to be exceeded in order to achieve and maintain good ecological status.

Main impact of the REBECCA lakes results on development and intercalibration of ecological assessment systems

The results presented in this special issue have had major impact on the development and intercalibration of ecological assessment systems for phytoplankton and macrophytes responses to eutrophication and for macroinvertebrate responses to acidification. These results have been particularly useful in the Northern countries, due to large datasets and more harmonised analytical methods than in other regions of Europe.

For phytoplankton, the type-specific reference conditions for chlorophyll now decided by the Northern GIG are largely based on the results reported in the article by Carvalho et al. (2008, this issue). Also the Central/Baltic and Mediterranean GIGs used input from this article and the underlying dataset on chlorophyll to decide on the reference conditions for the major lake types in these regions.

The Northern GIG countries extensively used data and results presented in Ptacnik et al. (2008, this issue) to examine the phytoplankton response to eutrophication for setting the high/good and good/moderate ecological status class boundaries. The thresholds identified in the response curves for major phytoplankton groups along the trophic gradient were used for the boundary setting (Fig. 4), and this procedure was found to be consistent with the normative definitions given in Annex V and further

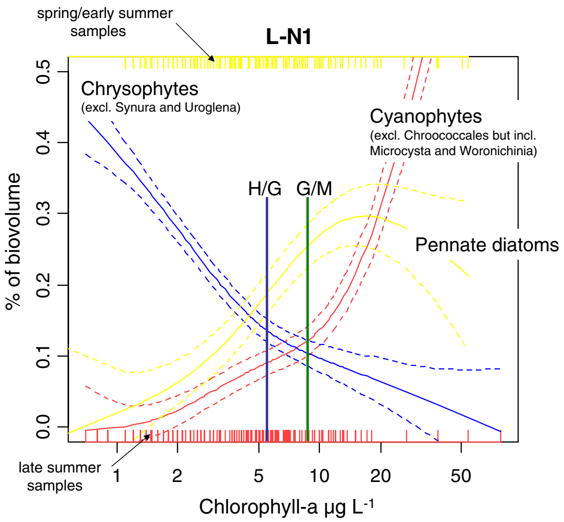

Fig. 4 Ecological status class boundaries (high/good (H/G): vertical blue line, and good/moderate (G/M): vertical green line) for the Northern lowland lake type L-N1 (moderate alkalinity, clear-water lakes) agreed by all countries in the Northern intercalibration group (NGIG) based on response curves for major phytoplankton group indicators, expressed as proportion of total biovolume (*y*-axis) along the trophic gradient, expressed by chlorophyll a (Chl-a, unit lg/l, *x*-axis). The response curves are based on results given by Ptacnik et al. 2008, this issue and the indicators are also explained in that article. Data on chrysophytes and Cyanobacteria are from late summer (July–September), whereas data on pinnate diatoms are from spring/early summer (May–July). The distribution of the data along the chlorophyll gradient is shown as red and yellow bars at the bottom and top of the diagram, respectively

elaborated by the Northern GIG (see EU Intercalibration report 2007). The phytoplankton dataset compiled in REBECCA for the Central/Baltic region have also been used by the Central/Baltic GIG in their discussions on metrics to use for classification and intercalibration of class boundaries, but the noise in the dataset made the applicability of the results less straightforward than for the Northern GIG.

The type-specific regressions between chlorophyll and TP given by Phillips et al. (2008, this issue) can be used to set class boundaries for TP based on the agreed intercalibrated chlorophyll boundaries set in the intercalibration by all GIGs. These type-specific regressions for different lake types will give more precise boundaries for TP than the more general models that have been extensively used for water management for several decades (OECD 1982). Setting the good/moderate class boundary for total phosphorus is particularly important in order to quantify the need for reduction of phosphorus loads to eutrophied lakes.

In order to set the good/moderate boundaries for macrophytes, the Northern GIG countries have used both the trophic index reported by Penning et al. (2008b, this issue), as well as supporting information from the sudden drop curves showing an abrupt decrease in relative abundance of sensitive taxa (e.g. large Isoëtids) above a certain threshold of TP (ca. 20–40 μg l^{-1}) (Penning et al. 2008a, this issue). Similar results found for charaphytes by Penning et al. (2008a, this issue) have also been used in the Central/Baltic GIG for discussions of class boundary setting for macrophytes in alkaline lakes. For this large region, however, numerous other metrics have been used by different countries for developing their classification systems. The comparability of these systems is debatable and intercalibration has been difficult (see Toth et al. 2008, this issue).

Also, data on response of benthic invertebrates to acidification (Schartau et al. 2008) have been extensively used by the Northern GIG countries to select relevant indicators for use in class boundary setting and intercalibration for this biological element.

The data and results relating to response of benthic invertebrates to eutrophication (O'Toole et al. 2008, this issue), and to response of fish to acidification (Hesthagen et al. 2008) have not been used for development and intercalibration of classification systems so far, mainly because these combinations of biological elements and pressures have not been addressed by the GIGs in the first intercalibration round that ended in late 2007. However, in the second phase of intercalibration, starting in 2008, more emphasis will be put on the remaining combinations of biological quality elements and pressures. New expert groups dealing with benthic invertebrates and fish in lakes have recently become established, and they include evaluation of REBECCA results in the planning of their future work.

Conclusion—The REBECCA project work on lakes have identified useful indicators and have quantified some of thresholds that are suitable for setting boundaries for good status. The project results have thereby made significant contributions to support the intercalibration of classification systems, as well as to support a more sustainable water management in Europe in the near future in compliance with the Water Framework Directive.

Gaps and future needs for research

The major gaps remaining after the REBECCA project in terms of relationships between biological elements and human pressures are first of all the responses to hydromorphological pressures, such as water level fluctuations, which are expected to increase also due to climate change. In REBECCA, ecological response to hydromorphological pressures was tested only to a very limited degree for lake macrophytes in Norwegian and Finnish lakes and reservoirs (Hellsten and Mjelde 2007). For other biological elements (macroinvertebrates and fish) there is so far less knowledge on large scale responses to hydromorphological pressures in lakes, although some case studies demonstrating effects on macroinvertebrates and fish have been reported (Richardson et al. 2002).

For lake eutrophication, there are still severe knowledge gaps, although most studies presented in this special issue deal with biological responses to eutrophication pressures. The remaining gaps primarily concern fish and macroinvertebrates, for which preliminary indicators are developed mainly for very shallow lakes (see Moss et al. 2003). These indicators need testing in other lake types, and new indicators may be needed. However, also for phytoplankton and macrophytes there are still community characteristics for which relationships have not been described in terms of large scale analyses for various lake types. These characteristics are frequency, intensity and duration of phytoplankton blooms and macrophyte abundance, such as maximum growing depth or % cover. Both these characteristics are included in the WFD Annex V and are necessary for a complete description of the responses of phytoplankton and macrophytes along the trophic gradient. For all biological elements, the final challenge is to find the best way to combine these different characteristics/metrics (abundance, community composition, other community characteristics) into a final assessment, either by constructing a multimetric index composed of single metrics, or by some other way of combining the different single metrics (Mischke and Nixdorf 2008).

Also, impacts of combined pressures (e.g. eutrophication and hydromorphological pressures, or acidification and hydromorphological pressures) are virtually unknown, despite the fact that combination of different pressures is often the real life situation in many lakes. The combined impact could potentially be larger (or smaller) than the sum of the individual impacts.

An additional research challenges is to evaluate the effects of measures taken to reduce the pressures on lakes and rivers. The response curves for the various biological elements to pressure reduction compared with pressure increase can be quite different due to hysteresis and resilience causing long delays in the ecological response, and the system may not turn back to the initial state unless the pressure is reduced way below the original threshold (see Fig. 5).

Local or regional extinction of species, caused by pressures such as acidification will also cause long time delays of recovery, and estimation of time needed for recolonisation of key species should also be included in future research.

Knowledge on the difference among the response curves for increasing versus decreasing pressures is important to allow monitoring of the effects of pressure reduction measures on the ecological status of the different biological elements and of the lake as a whole.

Another fundamental and urgent research challenge is to estimate the impact of climate change on the thresholds for setting the good/moderate class boundary, as well as on the baseline for setting reference conditions, since such research is essential for successful future water management. If these thresholds will occur at lower pressure levels under climate change than under present climate conditions, additional pressure reduction measures will be needed to reach the good status objective of the WFD. Existing climate scenarios predict higher temperature and changes in precipitation patterns in many regions of Europe, which will affect both underwater light climate, as well as other pressures. This will most likely affect the biological communities in a number of ways, for example by giving more favourable conditions for Cyanobacteria (Jöhnk et al. 2008).

Finally, perhaps the most important research need is to assess the uncertainty in the existing relationships and find ways to reduce them, through harmonisation of analytical and sampling methods,

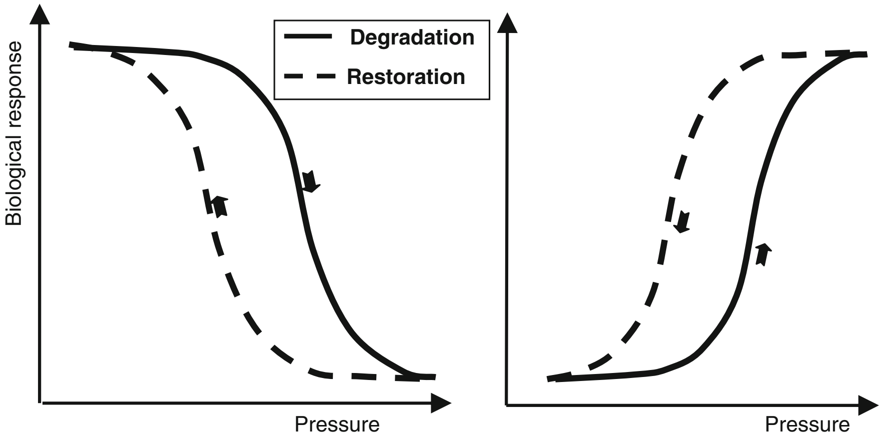

Fig. 5 Generalised biological threshold responses to pressure for a component decreasing with pressure (left, e.g. benthic fauna diversity vs. pH) and increasing with pressure (right, e.g. Cyanobacteria vs. TP). Degradation and restoration responses often follow different trajectories due to hysteresis and other time-lags

through sufficient sampling programmes, as well as through further research on ecological processes that are needed to improve predictive models. This is urgently needed to minimise the risk of misclassification of ecological status in European lakes.

These challenges and gaps should be given much attention in future research projects on ecological status assessment of water bodies.

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