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Abstract:
The report presents the papers and conclusions from the "Workshop on biological assessment and monitoring; evaluation and models", arranged by the International Cooperative Programme on Assessment and Monitoring of Acidification of Rivers and Lakes (ICP Waters) and the International Cooperative Programme on Integrated Monitoring (ICP 1M). The workshop was held in October 1998 in Zakopane, Poland. The workshop agreed upon the necessity of combining physico-chemical and biological monitoring. The importance of sampling at fixed locations and at fixed times were stressed, thereby minimizing “noise” in the database. The biological monitoring should include examination of all aspects of the biota and their interactions, but it is clear that a very good indication of the detrimental ecological effects, due to acidification, is obtained from studying the macroinvertebrate communities and fish in the context of critical loads. The biological monitoring should continue with the invertebrate studies. The importance of including fish was emphasized, using methods of testfishing, ecotoxicology and bio-accumulation of heavy metals and POPs. In addition, the benefits of using other elements of the biota such as diatoms, other algae and zooplankton were shown, as was the importance of food chain effects, illustrated by the work on waterbirds in Canada. The need for more work to be done in assessing the critical loads for different regions (within a variety of water qualities) was highlighted.

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Preface

The "Workshop on biological assessment and monitoring: evaluation and models", was arranged by the International Cooperative Programme on Assessment and Monitoring of Acidification of Rivers and Lakes (ICP Waters) and the International Cooperative programme on Integrated Monitoring (ICP IM). The organisation of workshops on important topics for monitoring is one of the main objectives for the Task Force programmes. Effect studies on aquatic communities is one such topic. The workshop was held in October 1998 in Zakopane, Poland.

The aim of this workshop was to give an overview of biological monitoring and the possibilities, advantages and disadvantages of using different monitoring strategies. The workshop covered different approaches from simple tolerance limit methods, the presence/absence methodology, to more advanced statistical techniques like multivariate statistical analysis. Through these methods a large number of environmental factors can be tested and their importance evaluated. The workshop evaluated the power of the different methods, as well as their strengths and weaknesses with respect to requirements of sampling, identifications, data handling and processing. The workshop sought to identify the most suitable methods for detecting trends in biological effects caused by acidification and also to discriminate between direct and indirect effects. With this in mind and knowledge about available recourses for monitoring, the question is to choose the most suitable method(s).

The Program Centres of ICP Waters and ICP IM are very grateful to Adam Woraztynowicz and Dorota Rzychon, Institute for Ecology of Industrial Areas in Katowice in Poland for arranging and hosting the workshop. The contribution of Liv Bente Skancke in editing the proceedings is gratefully acknowledged.

Oslo, July 1999

Gunnar G. Raddum
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1. Workshop report

Organised by: Gunnar G. Raddum, Norway
Chairpersons: Bjørn Olav Rosseland, Norway
Jim Bowman, Ireland

Participants from 15 countries.
Speakers from: Poland, Czech Republic, Latvia, Canada, Ireland, UK, Norway, France.

Summary

The workshop agreed upon the necessity of combining physico-chemical and biological monitoring. The biological monitoring should include examination of all aspects of the biota and their interactions, but it is clear that a very good indication of the detrimental ecological effects, due to acidification, is obtained from studying the macroinvertebrate communities and fish in the context of critical loads. The need to get preacidification or appropriate background data for the purpose of estimating, by comparison with current data, reductions in biodiversity due to acidification was highlighted.

The need to carry out monitoring in accordance with the methodologies recommended in the programme manual was stressed, as was the importance of sampling at the same fixed locations and times of the year. The value of the database was recognised and the multipurpose use of the database was encouraged.

Conclusions

The ICP Waters biological monitoring evaluates the ecological effects of airborne pollution. The workshop agreed upon the necessity of combining the water chemistry and biological monitoring. The biological monitoring should continue with the invertebrate studies. These organisms are the link between the chemical and physical environment and the higher trophic levels. The importance of the use of indices was evaluated and it was shown that by careful use of these indices the different levels of acidification could be accurately distinguished. The need to continue the work on the sensitivity of the different species used in the indices was stressed. Taxonomic richness related to pH was illustrated, and the high value of having information also on the quality of water not sensitive to acidification was pointed out.

Dependent on the different eco-regions and the chemical prehistory, the critical loads can be estimated and evaluated. In Norway, the critical load can be set to an ANC of 20, whereas in other areas a level of 50 is appropriate. The need for more work to be done in assessing the critical loads for different regions (within a variety of water qualities) was highlighted.

The importance of including fish was emphasised. The initial step, where appropriate, would be to gather data on fish populations by standard gillnet fishing according to the recommendations in the ICP Waters Programme Manual. In rivers and streams, ecophysiological methods are recommended, using a combination of blood sampling, histology and measurements of metal precipitation on gills. For these methods, existing protocols should be adopted from an ongoing EU research project (MORAL), and already used in some ICP Waters sampling points.

The workshop recognised the value of the databases for use in the other contexts, such as heavy metals, organic pollutants, climate change studies etc.
The ICP Waters database gives information about the biodiversity in the different regions. A further objective is to try to evaluate or get information on the preacidification status. Comparing this status with the existing situation thereby highlights the "gap" or the reduced diversity caused by acidification. This was clearly illustrated by the studies in the Vouges Mountains, France.

The importance of sampling at fixed locations and at fixed times were stressed, thereby minimising "noise" in the database.

In addition to macroinvertebrate and fish investigations, the benefits of using other elements of the biota such as diatoms, other algae and zooplankton were shown. In the Tatra Mountains, historical record of the zooplankton species structure was used to discover the acidification status of lakes. In the same area, concentration of total phosphorus (TP) and dissolved organic carbon (DOC) as well as phytoplankton and zooplankton biomass were closely related to acidification of the lakes. The importance of food chain effects were illustrated by the work on waterbirds in Canada. Invertebrates are the key element in the food chain. In fishless regions, the abundance of piscivore birds decreased whereas that of the macroinvertebrate eating birds increased in abundance. Reduced local emissions resulted in a significant improvement in water quality, and the reintroduced fish were soon to have a competitive advantage to the detriment of the macroinvertebrate eating birds.

Even in situations of water quality induced improvements in biota, the important position of the macroinvertebrates in the interaction between higher trophic levels was apparent. The value of understanding the process was underlined. In the course of lake recovery studies in Canada, unexpected oxygen depletion was found in the deep layers of some lakes during winter time, thereby affecting the biota. This illustrates the importance of connecting physical/chemical data to biological studies.
2. Large scale monitoring of invertebrates: Aims, possibilities and acidification indexes

Gunnar G. Raddum, University of Bergen, Norway

Introduction

The invertebrate community in freshwater consists of a large number of species living in balance with each other regarding food resources, competition, prey/predator relationship etc. All interactions caused by the organisms are called biotic or indirect factors. In addition, the physical and chemical environment or abiotic factors also affect the structure of the community. These parameters are named abiotic or direct factors. In all ecosystems the species composition is influenced by abiotic and biotic interactions and geographical distribution patterns of the organisms. Therefore, monitoring of invertebrate communities ideally should take into account all variables and evaluate the importance of the different parameters in relation to the observed community. This is usually costly and complicated, and the explanatory power of a single factor is often low in balanced ecosystems. On the other hand it is also possible to design monitoring with respect to special variables and investigate their importance. Usually, community changes start with a change in a physical or chemical factor that exceeds the tolerance range of an organism. An example is acidification, which leads to low pH and increased content of aluminium in lakes and rivers, making the water toxic for sensitive species (Raddum 1979, Matthias 1983, Engblom & Lingdell 1984, Økland & Økland, 1986 and Hermann et al. 1993). The toxicity, that is the direct effect, is easy to detect and separate from indirect biotic effects and by this suitable for monitoring purposes.

Monitoring strategies

Monitoring programmes can be divided in the following types:

1. Monitoring aiming to investigate the whole biota, or parts of it, to detect natural fluctuations in unaffected areas. These types of investigations are of basic nature and bring forward knowledge of the natural fluctuations. This information is necessary for effect evaluation of all manmade disturbances connected to industrial development, hydropower regulations, drinking water management, water enrichment and purification etc. Fundamental knowledge about the normal fluctuations makes it possible to detect irregular situations. As an example, information about the geographical distribution of invertebrates in Norway and other countries was the basis for detecting fauna damages, caused by acid water (Økland 1969, Hagen & Langeland 1973, Sutcliff & Carrick 1973, Almer et al. 1974, Scheider et al. 1975).

2. The other type of monitoring is limited, aiming at a specific task. This monitoring is applied in its nature, with defined objectives. The biological monitoring in the ICP-water program is of this character, but it also adds new general information about ecological demands of invertebrates and their distribution, to the general pool of knowledge.

The Manual for ICP-waters focuses on monitoring the extent of acidification, based on aquatic communities. For this purpose it relies on the presence/absence of common and widespread acid sensitive invertebrate species. The analyses result in a number, i.e. the acidification index (Raddum & Fjellheim 1984, Raddum et al. 1988, Fjellheim & Raddum 1990).
**Evaluation of data**

*The acidification index 1*

The database on aquatic invertebrates used for evaluation of acidified water in Norway goes back to 1976. The acidification index, hereafter named Index 1, was primarily based on water chemistry data and fauna from western and south-eastern Norway, but has later been evaluated for all parts of Norway. The Index 1 is based on the knowledge of critical limits for different species (Raddum 1979, Raddum and Fjellheim 1984, Raddum et al. 1988, Fjellheim and Raddum 1990) and a score is given for the invertebrate assemblage using a range from 0 to 1. The score 1 means that the locality contains one or more species with a low tolerance for acidic water (can not tolerate a pH lower than 5.5). If one or more of these species are found in a locality, there is no evidence of damage to the invertebrate populations due to acidification. A score of 0.5 indicates that all of the most sensitive species are absent, but the locality contains species that are moderately sensitive to acidification (can tolerate a pH down to 5.0). This type of community could be considered moderately damaged if acidification has occurred. An acidification score of 0.25 denotes that the locality lacks all the sensitive species mentioned above, but contains species with a tolerance down to pH 4.7. Localities containing only organisms with extremely high tolerance for acidic water (pH < 4.7) are given the score 0. Examples on invertebrates with different tolerance are given in Figure 1.

<table>
<thead>
<tr>
<th>pH</th>
<th>5.5</th>
<th>5.0</th>
<th>4.7</th>
<th>4.5</th>
<th>Category, acidification score</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td><strong>Very sensitive organisms, score 1</strong></td>
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<td></td>
<td><em>Lynnea peregra, Gammarus lacustris, Lepidurus arcticus, Baetis rhodani, Ephemerella vulgata, etc.</em></td>
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<td><strong>Moderate sensitive, score 0.5</strong></td>
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<td></td>
<td></td>
<td><em>Siphlonurus sp., Diura nansen, Isoperla sp., Apatania sp., Heptagenia sp.</em></td>
</tr>
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<td></td>
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<td><strong>Less sensitive organisms, score 0.25</strong></td>
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<td></td>
<td><em>Pisidium spp.</em></td>
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<td></td>
<td></td>
<td><strong>Very tolerant species, score 0</strong></td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td><em>Leptophlebia spp., Leuctra spp., Phryganea spp., Cyprinus flavidus, Corixidae, Coleoptera, Zygoptera</em></td>
</tr>
</tbody>
</table>

*Figure 1. Examples on invertebrates with different tolerance to pH modified from Fjellheim & Raddum (1990)*
The acidification Index 1 can be evaluated either from quantitative or qualitative samples. The sampling method must, however, ensure that the most common species are caught. The score does not take into account the number of species, species diversity or other information derived from the community in a locality, but only the presence/absence of acid sensitive species. In this manner the score will have the same meaning regardless of altitude and latitude. Furthermore, the score from different regions can be compared directly, synoptically or for trend evaluations.

To understand the advantage and weakness of Index 1 it is important to be aware of what happens with invertebrate communities during an acidification process. This is illustrated in Figure 2 where the different variables, both biotic and abiotic, are indicated by arrows acting on a community or a single species, the filled circle. In unacclimated areas the communities are in equilibrium with the variables, both biotic and abiotic. Acidity in water is one of the variables and when it increases the hydrogen ion and labile monomeric aluminum concentrations may exceed the tolerance limits for sensitive species. For all organisms these tolerance limits will be the main factor determining the presence or absence of the individuals. Other variables are at this stage of minor importance for the survival of the species. We know that increasing content of calcium and humic substances (brown water) to some extent can modify the critical limit. In the end the toxic effect will generate a new balance between the tolerant species through the biotic interactions.

Figure 2. Illustration of how a toxic variable (pH/Al) increases its importance regarding survival of a sensitive organisms.

When the level of acidity exceeds the critical limit for a species it will be the main variable determining the presence/absence of this species. At this stage we do not have to give much attention to other factors that normally influences the species, except for variables acting upon the tolerance limit. Through this it is possible to monitor the extent of acidification, dose-response, as well as trends
over time. This is a simplification and loss of general biological information will occur, but it will be sufficient for monitoring the development of acidification.

**Advantages of the presence/absence method**

Sampling method and number of samples is not critical as long as the recorded fauna is representative for the site. The real number of different species is not important, but their relative occurrence should be estimated from the samples. Evaluation of biotic interaction and effects other than acidity, is usually not important.

Direct comparison of results (acidification index) from different regions can be carried out. We do not compare the fauna composition itself, but the Index 1, which will arise from each fauna composition where species tolerances, critical limits to acid water, exists. Identification of the most common species is needed. Since the acidification index is based on presence/absence and not on number of sensitive species, it is enough that minimum one species characterising the acidification level, is correctly identified. This makes the system robust and allows assessments to be performed by scientists with varying degrees of taxonomic expertise. However, knowledge of the most common sensitive species in the regions is required and quality control of the taxonomic work is important.

**Acidification Index 2**

Unpolluted running water in Norway normally contains a high number of the very acid sensitive mayfly *Baetis rhodani* and several species of tolerant stoneflies. These species are living together in the same habitat. The number of individuals depends mostly on the productivity of the site and is quite variable. A difficulty arises in determining whether this variability in the number of *B. rhodani* is due to acid stress or other factors. To get information about this, the ratio between *B. rhodani* and the coexistent tolerant stoneflies at a site has been evaluated in relation to pH (Figure 3).

In clear running water with pH ≥ 6 the ratio between *B. rhodani* and tolerant stoneflies is ≥ 1 at sites situated below the timberline, defined at 650 m a.s.l. in the study of Raddum and Fjellheim (1984). When pH fall from 6 to 5.5 the ratio is in the most cases reduced to zero. It is suggested that the reduction in the ratio reflect sublethal stress on *B. rhodani* before extinction. This information is utilised in a modified acidification index. To discriminate between the original acidification index and the modified index they are named Index 1 and Index 2 respectively. Index 2 is valid only for clear water streams where *B. rhodani* is the only sensitive organism indicating score 1. Index 2 will make a smooth curve for the score between 0.5 and 1.

**Definition of Index 2:**

\[
\text{Index } 2 = 0.5 + \frac{\text{B. rhodani}}{\text{Stoneflies}}
\]

When the *B. rhodani*/Stoneflies ratio ≤ 0.5, Index 2 will be a number between 0.5 and 1. At ratios ≥ 0.5, Index 2 will be ≥ 1. In these cases the score is set to 1 so it can be compared with Index 1. Since Index 2 takes into account sublethal stress on *B. rhodani* the score value will be more conservative than the value based on Index 1. Examples on this are given in the result chapter.
Figure 3. Ratio between number of Baetis and stoneflies in relation to pH (after Radchun and Fjellheim 1984).

How to perform monitoring

Data stratification

Sampling method, habitat and sampling time are variables that influence the invertebrate composition of a sample. The sampling method employed is determined by the objective of the monitoring and the same method and sampling duration should be used throughout to minimise variability caused by these factors. Furthermore, site location and habitat type sampled should be fixed to reduce possible variation caused by sampling different locations.

The variation in lifecycle among invertebrates is highly variable. Due to this it is important to standardise sampling time. When choosing sampling season attention should be paid to the presence of easily detectable stages of important species.

The processing of the data should be constant, and the highest taxonomic resolution, the species level, should be achieved. This is necessary for the comparison and evaluation of species distribution in different regions.

What is possible and realistic in a large program?

In monitoring programs with many participants, the different laboratories will have various practice and competence. Since the data to be evaluated should be harmonised, attention must be given to what is possible to do among the participants. The crucial questions are resources with respect to economy, equipment and expertise, and which data that can be produced over a long time period.
Monitoring exercises in Norway.

The invertebrate monitoring in Norway has been performed since the beginning of the eighties. In river systems the number of sites have mostly been between 15 and 20, distributed in the tributaries and in the main river itself. The data will then give information about the situation in different parts of the watershed.

For investigating the status of lakes, we have standardised the sampling to the main inlet, one site at the shoreline, and in the outlet stream (Figure 4). By this we get information about the acidification status of the water entering the lake, the water in the lake and the water leaving the lake. The sites will cover both stream and littoral habitats, and makes it possible to evaluate the status based on different communities.

![Diagram showing sampling strategy for evaluation of the acid status of lakes.](image)

*Figure 4. Sampling strategy for evaluation of the acid status of lakes.*

Sampling has taken place during spring (two weeks in May) and fall (in September/ October) both for watersheds and lakes. Tributaries with “good” or “bad” water quality have been detected, as well as gradients in water quality within the watersheds.

Results from Norway

Examples of the use of Index 1 and 2 are given for a strongly and a less acidified watershed, and for a lined river. The indices are calculated as average values for spring and fall for all localities in the watershed.

The development of the acidification status since 1981 in the strongly acidified Farsund Watershed, situated in southern Norway, is shown in Figure 5. During the period from 1981 to 1994 no acid-sensitive mayflies were recorded in the watershed, and Index 1 and 2 were identical for these years. However, B. rhodani appeared in 1994 with a few individuals and Index 2 could be calculated.
Figure 5. Development of acidification index 1 and 2 in Farsund watershed. (Spring and autumn values are given for each year).

The presence of *B. rhodani* has been unstable and the species was not encountered in the spring of 1996 and 1997. The development of Index 1 and the sporadic recovery of *B. rhodani* during 1995 to 1997, indicates an improvement in the water quality compared with the period 1981 to 1993. The main change in the fauna composition is, however, the stabilised populations of moderate sensitive stoneflies and caddisflies at some sites.

The Nausta watershed on the western coast of Norway, while less acidified than the Farsund watershed (Figure 6), has a similar development in its acidification status. The occurrence of the most sensitive invertebrate species in the Nausta watershed was very unstable between spring and fall at the start of the monitoring period. In the period 1988 to 1992 the indexes were reduced principally due to heavy episodes of seasalt deposition.

Figure 6. Development of Index 1 and 2 in the Nausta Watershed. (Spring and autumn values are given for each year).
A seaweed episode in the winter of 1992 occurred along the whole coast of South Norway (Hindar et al. 1994) and is reflected in the acidification scores for the following spring in Nausta, Farsund (Figure 5) and Vikedal (Figure 7) Watersheds. Since 1994 Index 1 in Nausta has been 1 during fall, indicating low degree of acidification, while the springtime values ≈ 0.9 indicate acid stress at some sites. Index 2 shows lower values than Index 1, especially in spring. For unacidified watersheds both Index 1 and 2 should ideally obtain the score 1. Based on this, the situation in the Nausta Watershed is still unstable at some sites and subject to acidification. However, periods where Index 1 and 2 have been almost identical with value 1 have been observed, as in the fall of 1994 and 1996, indicating the potential for a fully recovered watershed as defined by the invertebrate assemblage.

The lower part of the Vikedal Watershed has been limed since 1987 with differing doses of lime and 
PH targets. In the first liming period, 1987-1990, the aim was to improve the water quality during 
spring to protect the smolt of the Atlantic salmon (Salmo salar) from acid water. After that period it 
was decided to lime the river throughout the whole year, with a pH target of 6.2 during the 
smoltification period. However, in 1994 the pH target was raised to 6.4. The effect of the liming has 
been evaluated by use of Index 1 and 2 (Figure 7).

![Vikedal watershed graph](image)

**Figure 7. Development of Index 1 and 2 in the limed Vikedal watershed. (Spring and autumn values 
are given for each year).**

The spring liming in 1987-1990 resulted in large fluctuations in the score of Index 1, indicating a very 
unstable presence of the most sensitive species over the year. For Index 2 the score was about the 
same for both spring and fall, demonstrating that the few recovered individuals of R. rhodani were 
under strong sublethal stress. The liming was far from successful during this period. The whole years 
liming until 1994 resulted in a strong variation between spring and fall both for Index 1 and 2. The 
seaweed episode in winter 1993 (Hindar et al. 1994), wiped out all the most sensitive species and Index 
1 and 2 became equal, showing the lowest score during the whole limed period. After increasing the 
PH target to 6.4 in 1994, Index 1 has been 1 during fall. Index 2 has increased and stabilised at a 
higher level. However, the liming does not fully protect the fauna, illustrated by the strong drop for 
both indexes in the spring of 1997.
Concluding remarks

The experience with the use of the acidification indices has been good with respect to the detection of acid episodes, and with trends in the acidification. Principally Index 1 can be used for all types of habitats, while Index 2 only can be used for running water. The indices are most applicable for common and widespread invertebrate communities with high turnover of species per year. It is possible to define 6 levels of damage by combining Index 1 and 2:

1. Index 1 = 0, strongly acidified
2. Index 1 = 0.25, seriously acidified
3. Index 1 = 0.5, moderately acidified
4. Index 1 = 1, little or no acidification
5. If Index 1 varies between spring and fall, or Index 2 is between 0.5 and 1, this indicates an unstable water quality
6. If the two indexes are 1 in both seasons, this indicates stable water quality with no harmful effect on sensitive species

When the focus is on toxic effects, it means that changes caused by biotic interactions are not accounted for. A careful evaluation of damage or change caused by acidification on the ecosystem can therefore not be evaluated by the mentioned score system. However, lists of species will be present and recovery or extinction of species can be detected and changes in species diversity can be described. Also a lot of different statistical analyses can be used on the material, but this has clear limitations, especially for analyses demanding quantitative samples. For the acidification score system, identification of sensitive species is important and must be stressed. However, if several sensitive species are present in the sample, misidentifications of one or a few species do not have to influence the score. In that respect the system has some robustness to the quality of the species identifications. This is not the case for multivariate techniques where a high quality must be obtained for the whole species assemblage, both sensitive and tolerant organisms. Quantitative techniques have, however, the potential to give better resolution than presence/absence methods.

The main questions to be asked when planning monitoring is therefore again:

- What is the purpose with the monitoring, and what is intended on the output level?
- What can be compared for small/large regions?
- What can be done with respect to costs, available equipment and personnel?
- What can be carried out for a long period of time without loss of comparability?

A monitoring program can both be too simple or too complicated or overloaded. The success of monitoring will depend on the questions asked above, and on a reasonable balance between the activities within the program.

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3. Strategies for fish monitoring at different environmental stress scenarios.

Bjørn O. Rosseland, NIVA, Norway

Introduction

The goal of the monitoring of fish populations in acidified areas is to describe the population structure in relation to pollution load of micropollutants (heavy metals and persistent organic pollutants (POP's)) in a way that acidification-induced changes in the chosen parameters can be recorded in a statistically appropriate manner. Therefore, a standardised sampling procedure is needed for site specific estimates of the variation of the chemical and biological parameters of interest.

The ICP Waters manual for fish population studies (Rosseland 1996), recommend the use of electro-fishing in running waters and gill netting in lakes with the "Nordic survey net" (Appelberg et al. 1995). Recent research and monitoring projects in running waters have used combinations of advanced in situ fractionation techniques for aluminium (Al) and ecophysiological and ecotoxicological methods to set water quality criteria for Atlantic salmon and brown trout (Rosseland et al. 1992, Lydersen et al. 1994, Pöleo et al. 1994, Krogland et al. 1998). In the EU-projects AL:PE 2 and MOLAR, sampling of i.e. live fish have also given new data on accumulation of micro-pollutants and histological effects on different organs in arctic and alpine mountain lakes (Rosseland et al. 1997a, Wathne et al. 1997, Wathne 1999). Results from these projects will be addressed, when a new monitoring strategy for streams and rivers in the ICP Waters localities is proposed.

Objectives for monitoring of fish populations

The precision of the monitoring of fish populations can be set at different levels depending on the chosen parameters and the intensity of the monitoring. A quantitative approach is emphasised for monitoring of age class composition, growth etc. when the number of lakes and rivers to be monitored in each country is relatively small (5-10). However, since the ecophysiological and ecotoxicological methods require detailed qualitative studies of individual fish, a combination of methods and approaches will be necessary.

The objectives of monitoring of fish populations are as follows:
1. Describe the fish population structure in acidified and non-acidified areas.
2. Provide data on early changes in reproduction
3. By repeated sampling - follow trends and changes in population size and structure in response to pollution load
4. Provide data for contamination loads (heavy metals, PCBs, PAH etc.)
5. Provide data for future monitoring or research projects

A fish is a long-lived animal, and can in some cases become more than 30 years old. Changes in yearly survival rate and exposure to accumulating micro-pollutants make the fish to an excellent early warning organism. A stronger focus on fish within ICP Waters, is thus recognition of this.
Problems for fish survival in freshwater and their relevance for monitoring

Even in neutral and non-polluted freshwater, the fish has to struggle for survival due to the fact that it is like "a bag of ions" swimming in a nearly "ion free" medium. Influx of water is therefore a permanent problem for keeping a stable body fluid osmolality of ca. 300 mOsm (ca. 75% being NaCl). With a freshwater osmolality of ca 0-10 mOsm in the surrounding freshwater, the mucus covered body, low permeability gill membranes and a production of a highly diluted urine, still can not compensate the loss of ions. An extremely effective set of ion pumps, partly located to the chloride cells of the gill membranes, involving enzymes like Na-K-ATPase, Mg-ATPase and carbonic anhydrase, maintain the homeostatic ion balance in the fish. One element, calcium, has a fundamental biological importance for water breathing animals and plays a key role in membrane permeability. Typically, lakes in the Norwegian high mountain areas with calcium (Ca) levels between 0.5-1 mg/L were the first to become barren during the early years of acidification (Henriksen et al. 1989). It has recently been demonstrated that under even more extreme low ionic conditions (non-acidified high mountain lakes), brown trout (Salmo trutta L.) can suffer from reproduction failure (Massabau et al. 1995) and reduced chloremia (low plasma chloride) if the water calcium is lower than 0.5 mgCa/L. The low chloremia seems to be species dependent, as Arctic char (Salvelinus alpinus L.) can compensate at the same Ca concentrations (Massabau 1999). Freshwater fish in its natural habitat, especially in low conductivity and low calcium waters, is therefore "struggling" for its survival, and any internal or external factor that influence the membrane functions or enzymes involved can disturb the ionic balance if not compensated effectively.

Chemical conditions affecting fish in acid waters

Mainly three elements, hydrogen ions (pH), aluminium and calcium, are still considered to be of most importance for the toxicity of acid water to freshwater biota (most recently reviewed by Rosseland and Starnes 1994 and Havas and Rosseland 1995). The effects of H+ and Al are dependent not only on animal species, but also on the life history stage of the animals and previous acclimation history. It is therefore a major challenge in the chemical monitoring of acid water, especially in running waters, to have a good enough resolution in time (sampling frequency) to be able to interpret biological responses.

In the field, the effects of Al alone are difficult to isolate from a variety of potentially interrelated adverse factors. During episodes of high water flow, and in lakes and streams where different water qualities mix, large variations in pH, Al-species, Ca and other ions and metals, and organic substances occur (Henriksen et al. 1984, Lydersen et al. 1994, Skogheim et al. 1984). When the pH of an acidic water body increases, for example when acid Al-rich water mixes with limed or neutral water, low molecular inorganic forms of Al are transformed to high molecular weight forms and hence precipitate. In such mixing zones, rapid Al-precipitation onto fish gills occurs, and combined with osmoregulation failure, inhibition of enzyme activities, and gill lesions, rapid mortality have been observed (Rosseland et al. 1992, Lydersen et al. 1994). Hence, water in the mixing zone is often more toxic than the original acid water (Rosseland et al. 1992, Poléo et al. 1994, Lydersen et al. 1994). In rivers having permanent areas with inequilibrium conditions, fish seems to avoid or have disappeared from such areas (Atland and Barlaup 1995).

The situation of inequilibrium in mixing zones, which is a time and temperature dependant phenomenon, calls for special in situ analytical techniques. With the traditional water sampling regimes where laboratory analysis was carried out days afterwards, one will not be able to characterise the water that was relevant for the biota at the time of sampling, since the in situ unstable water quality came to equilibrium in the bottle on the way to the laboratory. An understimation of the toxicity of the water can thus occur (Kroglund et al. 1998).
When discussing the effects of acid water, attention should also be paid to resistance mechanisms. On the individual level, the effects of the acid water can be resisted in a number of ways, such as through avoidance or escape reactions, by exclusion or removal (e.g. excretion of more mucus onto exposed surfaces or removal of mucus containing Al) or by repair of damage caused by the toxicant, see Calow (1991) and Rosseland and Staurnes (1994). Depending on the time between a stressful, but non-mortal exposure, and sampling and examination of fish, the levels found can thus have changed.

**Monitoring of fish populations in lakes**

A life history study of a fish population in a lake undergoing acidification, involves all the physiological mechanisms in the area of toxic effects and resistance. All temporary and/or long-term changes in water chemistry will have different impacts on individual fish and fish populations depending on the fish species, the life history stages represented, year class composition, the population size, spawning strategy and spawning facilities as well as competition between other fish species in the lake.

In freshwater fish populations, reproduction failure (i.e. reduced egg production or failure of survival of eggs and larvae) is still recognised as the main cause for fish population extinction (Rosseland 1986, Muniz 1991, Havas and Rosseland 1995, Hesthagen et al. 1999). The main cause of death for the early life history stages might therefore, from an ecological point of view, have the greatest impact on the environment. A monitoring based on multi- mesh sized gillnets, with the smallest mesh sizes able to catch one year old fish (1+), will be able to demonstrate early changes in reproduction failure leading to an ageing fish population (Rosseland et al. 1980, Figure 1).

![Figure 1. Test fishing results from two adjacent lakes in Southern Norway with populations of perch (Perca fluvialis L.). The perch population was still unaffected in Lake Djupedalsvatn (left) whereas the population in Lake Barkevatn (right) showed a clear sign of reproduction failure and ageing. Modified after Rosseland et al. (1980).](image)

Many of the now extinct fish populations in Southern Norway were overpopulated (stunted) before the negative effects of acidification started, with a typical decrease in condition factor with age and length of the fish. In the early phases of ageing, however, a reduced biomass (measured as catch per unit effort (CUE)) and a reduced competition for food for the remaining fish, lead to an improved condition and an increased condition factor with length (Rosseland et al. 1980, Figure 2). This response in condition factor can be used as an early sign of changes of population structure in a monitoring programme.
Figure 2. Catch per effort (C/E) in number of fish per gillnet series of brown trout in Lake Tveitvatn in the period 1976–1980 (top figure, modified from Rosseland 1981). In 1975, a massive fish kill occurred in the lake, and thereafter the population decreased and became extinct by the early 1980’s. The condition factor as function of fish length was typical for a stunted population in 1969, but changed gradually to a form typical for sparsely populated lakes (bottom figure, from Rosseland et al. 1980).

Relevance of water sampling at lake outlet to fish population status

The chemical monitoring of lakes in Norway is carried out by taking water samples at the lake outlet normally in autumn after lake turnover (Henriksen et al. 1988). The time perspective for changes in the fish population is years. Often the reproduction had failed many years before the water sample was taken. Moreover, the water chemistry representing the site for the toxicity of the most sensitive life stages will be at the site of reproduction (at certain depths in the lake or in spawning brooks), and not necessarily at the lake outlet. The lake water chemistry at the time of test fishing will therefore only be indicative for the relation to the fish response. The recognition of this is especially important when considering recovery from acidification. Depending on spawning area, a change to the better can come faster in a small catchment around a spawning brook than for the whole lake. Traditional water sampling might therefore in some cases underestimate the potential for recovery. On the other hand, a small catchment can be more vulnerable to episodic changes in water quality, i.e. during snow melt or flows during swim-up and start-feeding period. Due to this, an “overestimation” of degree of recovery based on lake outlet water sampling can thus occur.
Monitoring of fish populations in running waters – “episodic” exposed areas

Electrofishing
In rivers and spawning brooks a fish monitoring programme with the objective of building up a database describing community structure and density estimates for species of interest in certain sites or in the entire watersheds can be achieved by electro-fishing. The fish species composition and population density estimates can then be compared between watersheds or within a watershed over time. The samples describe the recruitment and the density of the youngest age-classes of the population. In addition to running waters, electro-fishing can be suitable also in lake littoral areas in order to complete the figure of fish communities obtained by gill netting. The methods for electro-fishing for monitoring purpose should follow recommendations in Rosseland (1996), which is based on the methods of Bohlin et al. (1989).

Ecophysiological and ecotoxicological methods
Electrofishing should, if possible, always be followed by a combination of water sampling and sampling of fish for ecophysiological and ecotoxicological analysis. “Traditional” water sampling gives only a momentary picture of the water quality at the time of fishing, and bear no information of the past, unless a more frequent water sampling programme is involved. However, by using in situ water chemistry analyses of aluminium for species fractionation and molecular size determination in combination with sampling of blood and gills, a better resolution of the past and present water quality can be found.

In River Sulldalslågen, an Atlantic salmon river on the West Coast of Norway, the link between the water quality and effects on Atlantic salmon population has been established. Electrofishing has been used to sample fish for characterisation of physiological and histological status, as well as to provide fish for experiments. Through channel experiments designed for “mixing zone waters” and in situ fractionation techniques for aluminium, and exposure of Atlantic salmon (Salmo salar L.) in cage experiments in the main river, blood physiology and gill histology have been used to set water quality criteria for the river (Kroglund et al. 1998, Finstad et al. 1999).

In Norwegian freshwaters, the most critical life history stage of fish is the smoltification stage of Atlantic salmon (Rosseland and Skogheim 1984), were the toxicity of inorganic monomeric aluminium (AlI or labile Al (LAI)) goes from 40-50 μgAl/L for salmon parr to 15-20 μgAl/L for smolts during the last two weeks of the smoltification period (prior to sea migration) (Staurnes et al. 1995, Kroglund et al. 1998). By using a 24hrs seawater challenge test, it was found that the most critical physiological process with the highest sensitivity to AlI was the ability to regulate seawater (Kroglund et al. 1998, Figure 3). A clear relation was found between increased concentration of total AlI (high and low molecular weight forms) in the water, and increased AlI precipitate on the gill tissue, reduced chloremia and increased mortality in freshwater and seawater (Figure 3).
Figure 3. Responses of Atlantic salmon (Salmo salar L.) smolts from River Suldalsløgen, South West Norway, to acid aluminium rich water, showing an increased Al precipitation onto gill tissue with increasing Al in the water at pH 5.8-6.3 (upper left), relation between gill Al concentration and plasma chloride (upper right), gill Al concentration and mortality in freshwater after 210 Hrs exposure (down left) and gill Al concentration and mortality after a 24 Hrs seawater challenge test (down right). After: Kroglund et al., 1998.

Marked changes in gill histology were noted that could be used as indicators (index A-D) of acidic and aluminium stress (Figure 4).
Figure 4. Gill structure (400X) of Atlantic salmon smolt from River Sulldalslågen exposed to different concentrations of aluminium in water at pH 5.8-6.3. The response is categorised as A-D: A normal, B moderate changes, C pronounced changes and D seriously changes. Abbreviations: pl, primary lamella; sl, secondary lamella; cn, blood vessel; el, epithelia lifting; m, mucus cell; e, epithelia covering adjacent secondary lamella; u, undifferentiated cells filling the inter-lamellar room between fused lamella. After: Kroglund et al. 1998.

River Sulldalslågen has a low concentration of organics (TOC = 0.5–2 mgC/L) particularly in spring, thus the chelating capacity for aluminium is low. In other rivers with more TOC, however, salmon smolt seems to tolerate a higher concentration of Al and more Al on gill tissue (Kroglund et al. 1999). This means that one has to establish river specific criteria for acidification and aluminium toxicity to fish if the water TOC differs within regions. This has also implications for both mitigation projects (liming) and for the models of recovery from acidification. Rivers with high TOC will have a lower target pH/Al/Ca than rivers with lower TOC, and they will probably have an earlier natural recovery in response to reduced depositions of S and N.

**Monitoring of micro-pollutants in fish**

The fish collected through a standardised test fishing program are extremely valuable as a source to evaluate the tissue concentrations of micro-pollutants like heavy metals and persistent organic pollutants (POPs). Since lakes in the ICP Waters programme should be negligibly affected by local sources of contamination, levels of pollutants in fish should thus reflect the long range transport of air pollutants as well as the in-lake biotic and abiotic factors which can influence the uptake of contaminants in fish. There are numerous reports on levels of micro-pollutants in fish. However, few programmes have selected lakes with fish populations that can be comparable and relevant for ICP Waters. Some results from two EU-Research projects, AL:PE 2 and MOLAR will be mentioned here. In AL:PE 2 (Wathne et al. 1997), one of the aims was to get an indications whether micro-pollutants in fish could be a relevant problem in alpine areas. As this was the case
(Rosseland et al. 1997a), the MOLAR project concentrated more work into fewer lakes to get a better statistical material. Results from MOLAR have not yet been published, but some of the conclusions will be referred to here.

ICP Waters lakes are interesting for studies of micro-pollutants in fish, as they are mostly low conductivity waters and some are located at high elevation and thus in low temperature regimes. This latter is important, as fish in arctic and alpine areas having a low annual mean temperature, can reach ages of > 20-30 years. Fish are therefore excellent integrators of pollutants.

The different micro-pollutants accumulate in different organs. To ensure that samples were collected comparably, a MOLAR fish training workshop was arranged, a video of the sampling procedures was made, and the sampling procedures were described in detail in a project manual (Rosseland et al. 1997b). Organs from single fish were divided, and sent to different specialist laboratories around Europe for analyses. This also included the ooliths for age determination, as a correct ageing of the individual fish is extremely important for evaluating the contamination levels.

**Heavy metals in liver and kidney and mercury in fish muscle**

Results from AL:PE 2 demonstrated clear differences in concentration of cadmium (Cd) and lead (Pb) in liver and kidney and mercury (Hg) in muscle of fish from the 17 lakes of AL:PE 2 (Rosseland et al. 1997a, Figure 5). Although some of the differences in trace metal concentration between fish were related to size (length, age, weight), principal components analysis (PCA) showed that the major variations are related to differences between sites and regions. Hg had highest concentrations in western localities close to the Atlantic Ocean whereas Pb and Cd showed low concentrations in the northern sites and highest levels in Central and Eastern Europe. On the whole, concentrations of Hg and Pb were below unacceptably high levels for human consumption. An exception was Schwarzschee ob Sölden in Austria where Pb exceeded the threshold. Threshold levels for Cd, on the other hand, were exceeded at many sites, especially those in the Alps and Polish Tatra Mountains (Rosseland et al. 1997a).

![Figure 5. Concentrations, +/- st. error, of cadmium (Cd) in fish liver from the various AL:PE sites, with no adjustments for differences in fish parameters or fish species. After Rosseland et al. (1997a).](image)

The importance of piscivore fish for biomagnification of mercury in food chains is well known. In high mountain and arctic lakes individuals of piscivore fish can get very old, and this is probably an important factor concerning the levels of mercury we observed in fish from Lake Arresjøen (Figure 6).
Figure 6. Mercury concentration in muscle versus age of individual fish sampled from a population of Arctic charr (Salvelinus alpinus L.) at Arresjøen, Spitsbergen, Norway. From: Wathne (1999)

The importance of defining the role of the individual fish in the food chain, can not be based on a stomach analyses, as this will only reflect the main food in the previous hours and days before catch. In MOLAR, muscle samples have been analysed for the isotopes of N14/N15. The relatively high fractionation rate of nitrogen through a food web can result in a difference in the isotope ratio of 0.3–5%. The relative distribution of nitrogen isotopes in different individuals, indicative of specific trophic classifications, can then be used as a continuous variable by which trophic position may be quantified. Isotopic measurements will therefore provide a time-integrated measure of assimilated diet and an independent means of evaluating the diet of the consumer. This is an important variable especially when detailed stomach analyses are impossible to obtain.

A fish programme in ICP Waters which will evaluate the levels of micro-pollutants, must therefore include nitrogen isotope analyses of fish muscle.

**Organic micropollutants**

The analyses of the organic micro-pollutants have demonstrated that both sediments and fish in Alpine lakes acts as sinks for these compounds. Fish muscle tissue from 14 AL:PE-2 sites were analysed for a range of organochlorinated compounds: hexachlorobenzene (HCB), total polychlorobiphenyls (PCBs), DDT derivatives (DDTs), and hexachlorocyclohexanes (HCHs) (Rosseland et al. 1997a). The results show a difference in geographic pattern across Europe between the organochlorinated compounds from industrial sources (PCBs and HCB) and those used as pesticides (DDTs and HCH). The former show only low variation in values between sites, whilst the latter show variations in values between two and three orders of magnitude with the highest values in Schwarzsee ob Sölden in the Austrian Tyrol. Overall a good relationship exists between the levels of pollutants found in fish tissues and the concentrations found in surface sediments. The values for fish are of the same order as those found in other aquatic systems in Europe not receiving direct discharges of organochlorinated compounds, indicating that these
compounds are easily transferred between ecosystems and that the atmosphere is an important transport medium.

In the MOLAR project, several new methods were used and more parameters analysed. In addition to muscle, liver and kidney for metal and POP's, the programme included blood samples for plasma ions, gill, liver and kidney tissue for histology, and bile for analyses of organic compounds (Rosseland et al., 1997b).

There is a positive correlation between low volatile PCB's and DDE in sediments and fish in relation to elevation and low temperature measured as period of ice-free season (Grimailt et al., 1999). This is especially interesting in relation to the Global Distillation Theory. Even Lake Øvrebø, west Norway, considered by the low metal accumulation in fish to be the most pristine and undisturbed lake in AL:PE 2 and MOLAR, is affected by POPs. It was found that the bile was especially rich in metabolites of PAH detoxification, which indicate an exposure to organic substances linked to industrial pollution (Escartín and Porte 1999).

The findings of significant levels of pollutants in fish from high mountain areas, even in regions considered pristine and unlikely to receive high levels of long range transported pollutants, calls for attention. A fish programme within ICP Waters should therefore include these compounds.

**Conclusion**

A monitoring programme for fish within ICP Waters should include several strategies:

**Lakes**

For lakes, which have a more stable chemical condition, the monitoring programme must include standard testfishing with the "Nordic series", as recommended by Rosseland (1996). The testfishing should be repeated at a frequency determined by the population status found in the individual lake, with the objective of picking up changes in recruitment and condition factor. The sampling and analyses programme should include tissue samples for determination of levels of micro-pollutants such as heavy metals and POPs. As these compounds are directly related to the socio-economic aspects and human consumption, it is of vital importance to follow standard procedures. The MOLAR manual (Rosseland et al., 1997b) is therefore recommended.

**Rivers and streams**

In rivers and streams, a traditional water sample describes the "at present" water quality conditions only. In important periods of the year such as the smoltification period in Atlantic salmon rivers, a high frequency sampling programme should be used. However, by using *in situ* water chemistry analyses of aluminium for species fractionation and molecular size determination in combination with ecotoxicological and ecophysiological methods, a much better resolution of the past and present water quality can be found. This will include sampling of blood for analyses of chloremia and other haematological parameters, and gill tissue for histological characterisation and determination of Al concentration.
References


4. Ordinations - some thoughts prior to computing

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Introduction

Ordination as a multivariate numerical procedure is related to the simultaneous analysis of many response variables. The ordination attempts to reconstruct a complex multidimensional space, defined by the species, in a simplified low-dimensional (axes) system that captures the major patterns in the data which may then be identified by the analyst, i.e. structuring the species composition along gradients using all the chironomids found in a series of lakes (Halvorsen 1999). Ordinations are one of many multivariate techniques that have gained a great popularity, mostly because of their efficiency in analysing complex biological data-sets and the interpretational simplicity of their graphical results (ter Braak 1994, ter Braak and Verdonk 1995). This popularity is reflected by the huge amount of papers published either discussing the technique or using it as a tool for furthering our biological understanding (see Birks et al. 1996, Birks et al. 1998). There are, however, very few papers that consider, in any depth, aspects of the analysis prior to computing the ordination analysis (Fortin et al. 1989, Jongman et al. 1995, ter Braak and Smilauer 1998).

A biological analysis that incorporates statistics may roughly be divided into three phases; (1) prior to computing, (2) the computing itself, and (3) after the computing. These three phases are closely linked and can not be considered independent of each other. Although the three parts have equal importance, the basis of a sound biological project is established in the phases prior to any computing. This is evident as the most advanced statistical technique and computer system will not be able to improve a poorly sampled biological data-set.

Prior to computing - the computing - after the computing

The phase of the analysis prior to computing includes the design of the project, applying for funding, establishing scientific partners, planning the field-work, doing the field-work, organising the observations for the computer, etc. (Jongman et al. 1995). Although there are many elements in any study-design, it can roughly be considered as (1) What are the biological questions we want to answer, and (2) How do we answer these questions? From this the sampling strategy follows, which should enable us to sample in such way that we collect the information that is needed to answer the biological question asked by using an appropriate statistical technique. The application of the sampling strategy during field-work results in observations, which subsequently are organised into a form readable by the computer, normally in a matrix form.

The following computing is an interactive process (Palmer 1993). The statistical analysis is constrained by which programs are available, and what statistical procedures we have knowledge about (Jongman et al. 1995). When the choice is to ordinate the data, the specific procedure undertaken is also dependent on the structure of the data, and how the data were sampled. The length of the gradient determines whether we should assume a linear relationship or unimodal relationship to the gradients (axes) extracted during the ordination, i.e. whether Principal Component Analysis (PCA) or a Correspondence Analysis (CA) is appropriate (ter Braak and Smilauer 1998). During the calculations many similar questions appear, such as: is it possible to do a direct gradient analysis? is it advisable to do one? (Jongman et al. 1995, ter Braak and Smilauer
1998). All such questions need answers which are related to the aim of the project prior to any computing.

Finally, when the results appear it is time to write papers and present the conclusions at meetings/symposia, etc. The post-computing part of a biological survey must consider how to interpret, infer, evaluate, publish, and present the results and conclusions based on the former parts of the analysis. Here the evaluation is of utmost importance to obtain a balanced study. It is an internal discussion of, for example, how to improve the total analysis, what key technical and biological knowledge did we gain, did we answer the questions of interest, and what do the results actually tell us?

Obviously there are many considerations to make as the project proceeds, and each of these must be thoroughly examined. In the following, some aspects of organising the observations for statistical analysis will be discussed, with emphasis on ordinations.

**The complexity of the model**

When observing the natural system of interest, i.e. lakes within a geographical region, it is our task to try to recapture the structure within the sampling unit in a language that is comprehensible for both the scientist and the computer. As biologists, we are interested in both the species and the environmental conditions that these species grow in. Both these are expressed as vectors of numbers or characters, which represent the amount or the quality of the appearance of that particular element. The observations for all sample units studied are thus systematised in a matrix form. To be able to detect reliably any trends that may be found within the data-set the number of sample units must be as large as possible.

In experimental procedures the number of observations is controlled by the number of replicates. However, in natural systems such replicates may be hard to obtain by. Thus, in field-studies, it is often more advisable to seek a representative sample of the natural system, i.e. describe the different possibilities present within the study area by repeated sampling along the target gradient. Therefore to gain a representative sample of a natural system, the number of sample units should be related to the complexity of the system, or in practice to the complexity of the model to be estimated.

This is best visualised by thinking of a constrained ordination where the species composition may be explained by several environmental variables. If the environmental variable of interest holds two possibilities (presence or absence of shade), then the variation within both these categories must be sampled. However, if the variables are continuous, as a pH-gradient from 4 to 7 with 0.1 units resolution there are 30 possibilities. If we should adequately sample the variation for all these 30 levels, the number of samples would be impractical. However, rather than studying each possibility we try to capture the variation of the response (here species composition) along such a gradient. Although, this reduces the sampling effort, the recommended number of samples is still high. As a rule of the thumb, one should not be pragmatic when discussing number of samples needed, but 50 samples along one gradient ought to capture the main trends in the response variables. As the number of predictors increase, increasing complexity of the model, the number of samples needed also increase. Two continuous variables with 30 levels each have a combined number of possible outcomes of 900 levels. Thus, for field studies the number of predictor variables (the complexity of the model to be) may indicate the number of samples that are needed to obtain a data-set that captures the variation within the natural system studied. However, for predictor variables that have a causal correlation this may be an overestimate, as some of the numerical options will not be possible in nature. Thus, the parameters used must be considered together when deciding on the number of samples that are needed to obtain a reasonable representative data-set. The complexity of a model is also related to the resolution we want to study the system in. If the response variables are wished studied at high resolution along the gradient then an increase in the number of samples...
is needed relative to studies of linear overall trends. Detection of high resolution trends often requires the use of polynomial terms (x^2, x^3, etc.) which represents independent dimensions in the model and thus an increased number of samples is needed.

In addition, in biological surveys it is often time consuming and expensive to cover various gradients, therefore many predictor variables are measured for a few lakes. Such fat data-sets (few observations relative to the number of species or predictor variables) will not yield a proper representation of all these variables. In addition, there will inevitably be a high correlation between some of the predictor variables, which may seriously weaken any inferences from such data. For example, in an ordination a relative high number of predictor variables in a constrained ordination (CCA = Canonical Correspondence Analysis) will ruin the effect of the predictors and the results will approximate those from a Correspondence Analysis (CA).

It is recommended that prior to the fieldwork, a few environmental variables of special interest are selected. Then during the fieldwork, one can ensure that the range for these variables within the study area is representatively and adequately sampled. However, in addition to these variables, one may obtain information on other variables. This is to study their complimentary effect or to see whether these alternatives may be more influential on the species composition.

Assumptions

All statistical procedures have a number of assumptions which are the criteria under which that particular test or procedure must be true. If violations of such assumptions appear, there can be no conclusions about the statistical significance of the relationship tested (Sokal and Rohlf 1995).

A recurrent assumption is the independence of the observations, i.e. the observations made at a particular sample site are not to be influenced by the situation at other sampling sites. The samples are related to each other through geographical and temporal space, thus the dependency-assumption is related to these dimensions. It is therefore of importance to record both when and where the sampling occurred. A violation of the assumption of independence will lead to a situation when we will more often than expected accept a false alternative hypothesis, i.e. we will often conclude falsely that there is a relationship between the variables tested (a high probability of a type I error).

The problem of such dependency can be minimised by having an even distance in time and space between the sample units (Palmer 1988, 1992). However, aquatic scientists working with between-lake studies have a limited possibility to select the position of the lakes. In any case it is always advisable to report the spatial and temporal (relative) position of the sampling units. The position of the sites may help us to evaluate the potential influence of such spatial and temporal dependency, and there are numerous ways of using such extra information. It may be used as a correction in statistical testing, i.e. a constrained randomisation test where the spatial or temporal structure is in general kept intact (ter Braak and Smilauer 1998). This will contribute to a more rigorous test and more reliable conclusions. Another procedure suggested is a partial analysis (Borcard and Legendre 1994, Legendre 1993, ter Braak 1987, 1988, ter Braak and Prentice 1988). The space/time structure is then incorporated into the estimation by the effect of these variables being allowed for. The analysis of the environmental variables is related to the residuals of the analysis of the species composition to the spatial/temporal trends (Legendre 1993).

Despite potential compensation for such dependency, it is often the direct focus of investigators (Austin 1977, Heegaard 1999, Legendre and Fortin 1989, Philippi et al. 1998). How do species composition change in time and does this correlate with a particular environmental factor? A common procedure is then to analyse the trend with the time as a predictor variable, and test the significance with modern randomisation procedures (see Philippi et al. 1998, Burrough 1995). However, such analyses must be interpreted with great care, and the statistical procedure used must be conservative.
The spatial dependency can be caused by biological contiguous processes, such as dispersal, migration, etc. But it can also be created by regional sampling. Large projects often involve different scientists visiting the different sites. If there are slight differences in their sampling procedure this may create a regionally correlated systematic error, which may be detected as a spatial process and this will then increase the probability that at the regional scale the statistician will accept a relationship that is not biologically true. It is therefore of extreme importance that there is a commonly agreed and detailed protocol describing in detail the sampling procedure and the time when the sampling should occur. The key to avoid such tedious problems is that all participants use the same technique to obtain the data, and that they sample at a similar time, either relative to an event or close to an exact date.

The quality of the data

The quality of the data must be high if there shall be any reliability for the results in any project. For scientists familiar with multi-institutional projects it is obvious that the same biological language is often not spoken. Therefore the homogenisation of the taxa involved is of utmost importance. An unharmonised species data-set will include uncontrollable bias and is likely to result in spurious conclusions. In the process of harmonisation, several decisions are required. The names used should include the same variation, i.e. the individuals identified are labelled at the same taxonomical level and given the same name by all investigators.

The ordinations use the species distribution and the species optima to structure the species composition along gradients (Figure 1a). Therefore the ordinations assume that their estimator, whether parametric or non-parametric, gives a good approximation of the optima and distribution of each taxon. Thus, it is of importance that the taxa identified do fulfil such assumptions of the estimator. This is also a necessity when the artificial taxon represents an agglomeration of two distinct taxa. For example, a biomodal response may contribute to a reduction in the between-variation as the procedure will estimate the optimum of that taxon to be the mean of the optima of the individual taxa (Figure 1b). This will underestimate the difference between the groups of sites that these taxa occur in.

To ensure the quality of the data it is also important that a taxon is assigned to only one category. Thus if species agglomerations (Leptod. spp.) are used, it should not include any of the taxa of the same genus that are identified to the taxonomical level of a species. (Species of Leptod nigra should not also appear in the level of L. spp.). However, this may be difficult as some specimens may be badly preserved and identification of these individuals is only possible to a level between the two taxa. Then an artificial agglomeration may be used for these two taxa. Such a group should then incorporate the doubtful individuals of both these taxa, but no other taxa of the same genus. However, in some cases doubtful specimens may belong to many taxa and/or the insecurity is may be between different pairs of taxa. In such cases it may be advisable to group all non-identifiable specimens into one taxonomical group, and treat them as one taxon. The influence of such grouping may be evaluated by comparing the results of the former analysis with the results with the whole genus as one taxon.

As the species are harmonised, it is equally important to harmonise the scale at which their occurrences are reported (Table 1). Obviously there are differences in interpretation between frequency, percentage and presence/absence data but a computer regards such numbers as they were of the same scale. In addition, the percentages may be from different totals which can also create interpretation differences. If different scales are used, they must be standardised to the same common “denominator”, which in most cases will reduce the sensitivity of the data but will increase their reliability. The latter will otherwise be lacking. Often such a common “denominator” is whether a taxon is present or not. This is a crude scale but it has a high reliability if the taxa are
harmonised. Therefore if a high sensitivity and reliability are needed for the analysis, the proper scale must be agreed upon prior to the fieldwork.

In addition to the harmonisation of the taxa and the scale that these are recorded in, it is equally important to harmonise and standardise the sampling of the predictor variables. Otherwise systematic errors may appear and the interpretation of the data becomes obscure.

Conclusions

As a small concluding remark it is vital for any project that all parts are discussed and considered prior to sampling and computing. Otherwise a poor start to the project will be the result, which will always lead to a low-quality end to the project. In comparison, a sound foundation for the project will help the scientist during the project to make reasonable decisions and actions.

Here I have only mentioned a few points that I think are important, and which, unfortunately, can be easily forgotten by scientists. In addition to these remarks, there are numerous other options in a biological survey that need at least equal or more consideration. The best way to create reliability in a project is to remember what, why, and when. What to do, why do that, and when to do it!

Acknowledgement

I would like to thank H. J. B. Birks and John-Arvid Grytnes for comments and kind corrections of my English.

References


Heegaard, E. 1999. The distribution of Uota crispa at a local scale in relation to both dispersal- and habitat-related factors. Lindbergia. (Accepted).


5. Comparison of different indices used in the 95 national survey of lakes and streams in Sweden.

Lars Eriksson, Dept. of Environmental Assessment, Sweden

Introduction

A national survey of benthic fauna took place in Sweden in the autumn of 1995. Some 700 streams and 700 lakes of different size were randomly selected for sampling. Samples were collected by the standardised kick-sampling technique and using a handnet with a mesh size of 0.5 mm. In streams sampling was carried out in riffle areas and in lakes sampling was carried out on exposed shorelines with hard bottom (0 – 1 m depth) without vegetation. However due to an early winter, sampling could only be carried out in 537 lakes and in 698 streams.

The lakes and streams sampled were well distributed across Sweden, indicating good measures of existing gradients of latitude, longitude, temperature and altitude (Figure 1). Lakes and streams affected by a) point sources of liming, b) acidification, c) agricultural activity (>20% of the catchment area was classified as arable land), d) sampling error, were withdrawn from the dataset. 364 lakes and 558 streams were left for analyses.

When analysing the material emphasis was placed on the indices performance along pollution gradients, and on selecting a group of metrics with relatively low redundancy (i.e. the metric selected should give relatively independent measures of stress). For four acidity indices the frequency of type I and II errors were also calculated.
Figure 1. Distribution of (a) lakes (n=537) and (c) streams (n=696) sampled in the 1995 national lakes and stream survey, and reference (b) lakes (n=364) and (d) streams (n=439) where sites affected by point-source pollution, timing, acidification (exceedence>0), agriculture (>20% of catchment classified as arable land) or sampling error (county of Västernorrland) were excluded. The lines denote five major ecoregions of Sweden.
Indicator metrics

A number of indicator metrics were considered for use in the classification of the ecological quality of Swedish lakes and streams using macroinvertebrates. In the selection of indicator metrics, emphasis was given to selecting metrics that can be used to assess general ecological quality as well as metrics that are considered more pollution-specific (i.e. metrics for assessing the effects of acidification and organic pollution or eutrophication stress).

**General ecological quality**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>abundance</td>
<td>Simpson (1949) and Shannon (1948)</td>
</tr>
<tr>
<td>diversity</td>
<td>Lenat (1988)</td>
</tr>
<tr>
<td>EPT</td>
<td>Armitage <em>et al.</em> (1983)</td>
</tr>
<tr>
<td>BMWP</td>
<td>Armitage <em>et al.</em> (1983)</td>
</tr>
<tr>
<td>ASPT</td>
<td>Moog (1995)</td>
</tr>
<tr>
<td>functional feeding groups</td>
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**Acidification**

<table>
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<th>Reference</th>
</tr>
</thead>
<tbody>
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<td>acidity index I</td>
<td>Raddum and Fjellheim (1984) or Raddum et al. 1988</td>
</tr>
<tr>
<td>acidity index II</td>
<td>Lingdell <em>et al.</em> (unpubl.)</td>
</tr>
<tr>
<td>acidity index III</td>
<td>Bakken and Aanes (1990)</td>
</tr>
<tr>
<td>acidity index IV</td>
<td>Henrikson and Medin (1986)</td>
</tr>
<tr>
<td>Baeotis/Plecoptera (ind.)</td>
<td>Raddum and Fjellheim (1984)</td>
</tr>
<tr>
<td>Ephemeroptera/Plecoptera (ind.)</td>
<td>Raddum and Fjellheim (1984)</td>
</tr>
<tr>
<td>Ephemeroptera/Plecoptera (taxa)</td>
<td>Raddum and Fjellheim (1984)</td>
</tr>
</tbody>
</table>

**Organic pollution**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saprobien index</td>
<td>Moog (1995)</td>
</tr>
<tr>
<td>Danish stream fauna index</td>
<td>Skriver <em>et al.</em> (1999)</td>
</tr>
</tbody>
</table>

The density (or abundance) and biomass of macroinvertebrate populations and communities are often used in environmental assessment studies.

Richness measures, such as number of taxa present at a site, are simple to calculate and are regarded as reliable indicators of environmental stress.

The EPT index, a form of the taxa richness approach that consists of the number of taxa from the insects groups Ephemeroptera, Plecoptera and Trichoptera.

Diversity indices relate taxon richness to abundance, and are frequently used in bioassessment studies.

Macroinvertebrates can also be divided into functional feeding groups.

Acidity indices are based on the known sensitivities of different taxa to pH. The difference between the indices are that some are based on species and some on species groups and on a compositional index (Henrikson and Medin 1986). There are also the ratios between the acid sensitive *Baetis* to the acid tolerant Plecoptera and acid sensitive group Ephemeroptera to Plecoptera.

The saprobien index of Moog (1995) has five saprobic categories descriptive of clean water to extremely polluted.
The Danish Stream Fauna Index considers not only the sensitivity of indicator taxa to organic pollution, but also takes into consideration the diversity of the community.

The BMWP and ASPT indicator metrics were originally developed in the United Kingdom for classification of organic pollution effects. The metrics use binary (presence/absence) data and taxonomic resolution is restricted to family level. In the BMWP score, values range from one to ten, with pollution tolerant families having low values. ASPT is BMWP divided by the number of families giving the BMWP score.

Table 1. Correlation (Spearman) of selected indicator metrics for riffle communities with metrics for acidification (exceedence > 0 and pH) and organic pollution (SNO₂⁺-NO₃ and TP) stress. Data are taken from RI95 (5 standardized kick-samples), limed sites and sites situated in Västernorrland were excluded from the analysis. In correlation between indicator metrics and chemical metrics for acidification stress (i.e. exceedence and pH), sites affected by agriculture (> 20 % catchment classified as arable) were excluded. Similarly for comparison with metrics for organic pollution stress (SNO₂⁺-NO₃ and TP), sites affected by acidification (exceedence > 0 or pH < 5.5) were excluded.

<table>
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</thead>
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<td>pH</td>
</tr>
<tr>
<td></td>
<td>(meq/m²/yr)</td>
<td></td>
</tr>
<tr>
<td>taxa richness</td>
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<td></td>
</tr>
<tr>
<td>abundance (CPUE)</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>EPT (taxa)</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>EPT (ind)</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Shannon’s diversity</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Simpson’s diversity</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>no. shredders (taxa)</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>no. grazers (taxa)</td>
<td>*****</td>
<td></td>
</tr>
<tr>
<td>no. detritivores (taxa)</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>no. predators (taxa)</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>BMWP</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>ASPT</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>acidity index I</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>acidity index II</td>
<td>****</td>
<td></td>
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<tr>
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<td>****</td>
<td></td>
</tr>
<tr>
<td>acidity index IV</td>
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<td></td>
</tr>
<tr>
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<td></td>
</tr>
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<td>****</td>
<td></td>
</tr>
<tr>
<td>Plecoptera (taxa)</td>
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<td></td>
</tr>
<tr>
<td>Plecoptera (ind)</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>Saprobiens index</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Danish stream fauna index</td>
<td>***</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001
Table 2. Correlation (Spearman) of selected indicator metrics for littoral communities with metrics for acidification (exceedence \( \geq 0 \)) and pH and organic pollution \((\text{SO}_{2}+\text{NO}_{3})\text{ and TP}\) stress. Data are taken from R195 (5 standardized kick-samples), limed sites and sites situated in Västerbotten were excluded from the analysis. In correlation between indicator metrics and chemical metrics for acidification stress (i.e. exceedence and pH), sites affected by agriculture (\( \geq 20\% \) catchment classified as arable) were excluded. Similarly, for comparison with metrics for organic pollution excluded.

<table>
<thead>
<tr>
<th>acidification</th>
<th>organic pollution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>exceedence</td>
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<tr>
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<tr>
<td>abundance (CPUE)</td>
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<tr>
<td>EPT (ind)</td>
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</tr>
<tr>
<td>Shannon's diversity</td>
<td></td>
</tr>
<tr>
<td>Simpson's diversity</td>
<td></td>
</tr>
<tr>
<td>no. shredders (taxa)</td>
<td>***</td>
</tr>
<tr>
<td>no. grazers (taxa)</td>
<td>****</td>
</tr>
<tr>
<td>no. detritivores (taxa)</td>
<td>***</td>
</tr>
<tr>
<td>no. predators (taxa)</td>
<td>****</td>
</tr>
<tr>
<td>BMWP</td>
<td></td>
</tr>
<tr>
<td>ASPT</td>
<td>**</td>
</tr>
<tr>
<td>acidity index I</td>
<td>****</td>
</tr>
<tr>
<td>acidity index II</td>
<td>****</td>
</tr>
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<td>acidity index III</td>
<td>****</td>
</tr>
<tr>
<td>acidity index IV</td>
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</tr>
<tr>
<td>Boeotis/Plecoptera (ind)</td>
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<td></td>
</tr>
<tr>
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</tr>
<tr>
<td>Ephemeroptera/</td>
<td></td>
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<tr>
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<td>Saprobia index</td>
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<tr>
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<tr>
<td>index</td>
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* \( p < 0.05 \), ** \( p < 0.01 \), *** \( p < 0.001 \), **** \( p < 0.0001 \)

Tests of the predicted response of the indicator metrics along pollution gradients showed a number of metrics to be reliable indicators of stress (Tables 3 and 4). Similar to correlation, pollution-specific indices generally performed better than metrics of general ecological quality. However, differences were found to exist between streams and lakes. For example, all of the metrics tested for acidification stress in streams showed the expected response (seven of seven), compared with only four of the seven metrics tested for lakes. For organic pollution, both of the metrics tested showed the expected response in streams, whereas only the Saprobia index showed the expected response for lakes. That the majority of indices tested were developed for running waters and not for lake-littoral communities can undoubtedly explain part of these results. In addition, these findings indicate that littoral communities may be used for monitoring and assessment of acidification stress, but are less reliable for monitoring organic pollution effects.
Table 3. Predicted response to pollution of selected indicator metrics of riffle habitats and results of test to distinguish pollution categories (Wilcoxon test). Data are taken from RI93 (5 standardized kick-samples), limed sites and sites situated in Viltornorrfärd were excluded from the analysis. Test criteria for acid stress; 2 classes (pH < 6 and pH ≥ 6.0) and for organic/eutrophication stress; 2 classes (TP < 25 and TP ≥ 50 µg/L). In tests between indicator metrics and metrics for acidification, sites affected by agriculture (> 20 % catchment classified as arable) were excluded. Similarly for comparison with organic pollution, sites affected by acidification (exceedance > 0 or pH < 6) were excluded. (n/a = not applicable).

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<th>organic stress</th>
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</tr>
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<tr>
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<tr>
<td>ASPT</td>
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</tr>
<tr>
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<td>No. grazers (taxa)</td>
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</tr>
<tr>
<td>No. detritivores (taxa)</td>
<td>variable</td>
<td>yes (-)</td>
</tr>
<tr>
<td>No. predators (taxa)</td>
<td>variable</td>
<td>no</td>
</tr>
</tbody>
</table>

Acidification

| acidity index I | decrease | yes | **** | n/a | - |
| acidity index II| decrease | yes | **** | n/a | - |
| acidity index III| increase | yes | **** | n/a | - |
| acidity index IV | decrease | yes | **** | n/a | - |
| Baetis/Plecoptera (ind) | decrease | yes | **** | n/a | - |
| Ephemeroptera/ Plecoptera (taxa) | decrease | yes | * | n/a | - |
| Ephemeroptera/ Plecoptera (ind) | decrease | yes | **** | n/a | - |

Organic pollution

| Saprobien index | increase | n/a | - | yes | **** |
| Serbian stream fauna index | decrease | n/a | - | yes | **** |

* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001
Table 4. Predicted response to pollution of selected indicator metrics of littoral habitats and results of test to distinguish pollution categories (Wilcoxon test). Data are taken from RI95 (5 standardized kick-samples), limed sites and sites situated in Västernorrland were excluded from the analysis. Test criteria for acid stress; 2 classes (pH < 6 and pH ≥ 6) and for organic/eutrophication stress; 2 classes (TP < 25 and TP ≥ 30 µg/L). In tests between indicator metrics and metrics for acidification, sites affected by agriculture (> 20 % catchment classified as arable) were excluded. Similarly for comparison with organic pollution, sites affected by acidification (exceedance > 0 or pH < 6) were excluded. (n/a = not applicable).

<table>
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<tr>
<th>Predicted response</th>
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<td>*</td>
<td>no</td>
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<tr>
<td>abundance (CPUE)</td>
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<td></td>
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<tr>
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<td>**</td>
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</tr>
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<td>*</td>
<td>no</td>
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<td>*</td>
<td>no</td>
</tr>
<tr>
<td>Simpson’s diversity</td>
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<td>*</td>
<td>no</td>
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<td></td>
<td>no</td>
</tr>
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<td></td>
<td>n/a</td>
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<tr>
<td>Plecoptera (ind)</td>
<td>decrease</td>
<td>no</td>
<td></td>
<td>n/a</td>
</tr>
</tbody>
</table>

| Organic pollution |                              |   |                               |   |
| Saprobiën index   | increase                     | n/a | -                            | yes | * |
| Danish stream fauna index | decrease       | n/a | -                            | no  |   |

* p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.00
Type I error or is the probability of rejecting the null hypothesis when it is in fact true; the type II error is the probability of failing to reject the null hypothesis when in fact it is false. The mean frequency of type I errors was higher for lakes (36% for both exceedence and pH) than for streams (15-16% for both exceedence and pH) (Figure 2). In contrast, lakes exhibited an overall lower frequency of type II errors (16% for exceedence and 4.3% for pH) compared with streams (44% and 16%) (Table 3 and 4). However, relatively large differences were noted among the four acidity indices tested here. For exceedence as a measure of acidification stress, acidity index II had the lowest frequency of type I errors for both streams (10%) and lakes (24%) (Table 3). Acidity index IV, on the other hand, had the highest frequency of type I errors for both streams (23%) and lakes (43%), but this index also had the lowest frequency of type II errors (27% for streams and 11% for lakes). Also, for pH as a measure of stress, acidity index II showed the lowest frequency of type I errors (9.7% for streams and 24% for lakes), and acidity index IV had the highest (22% and 43%) (Table 4). Acidity indices II and IV had the lowest frequency of type II errors, i.e. 0% and 13% (streams) and 4% (lakes), respectively.

Figure 2. Frequency of type I and II errors of four acidity indices. Type I error or (a) is the probability of rejecting the null hypothesis when it is in fact true; the (b) or type II error is the probability to fail to reject the null hypothesis when in fact it is false.

The overall high frequency of both type I and II errors is alarming and this finding deserves greater attention. Increasing the number of indicator taxa used in the metrics may result in a lower frequency of type I errors. This conjecture was supported here; acidity index II which uses 146 taxa had lower type I errors frequencies than acidity index I (n = 43 taxa) and III (n = 30). However, of greater concern in biomonitoring is the relatively high frequency of type II errors, in particular as these indices are widely used in the Nordic countries. Careful consideration should be given to the tolerance levels assigned to the individual taxa (i.e. taxa incorrectly classified as "sensitive" will result in a type II error). In addition, the use of more composite indices (like acidity index IV) is seemingly a more robust approach, resulting in lower frequencies of type II errors.
References


6. Acidification in the Vosges Mountains (North-eastern France): Assessment of biological status by the use of a multimetric approach

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Abstract
Numerous headwater streams are still acidified in the Vosges Mountains (N-E, France). In order to assess the biological status of such streams, a multimetric study was performed at different organisational levels of the ecosystem.
A survey of macroinvertebrate communities of 41 streams clearly demonstrated an important erosion of biodiversity in acidified streams. The Biological Index of Acidification (BIA) based both on the acid-sensitivity and on the taxa richness may represent a useful and efficient tool to monitor acidification.
The study of leaf litter breakdown under different acidic conditions showed that the eradication of efficient shredders such as Gammarus may lead to a drastic dysfunctioning of acidified ecosystems by reducing the decomposition rate of allochthonous materials which represents a major source of energy in headwater streams.
At a lower organisational level, the study of some blood parameters of transferred brown trout revealed that after 48 h of exposure under acidic condition, trout were face to a depletion in Na and Cl plasma concentrations. However, autochthonous trout obtained from slightly to moderately acidified streams did not suffer of any ionic regulatory failure showing that fish may acclimate to such conditions.
The study of the feeding activity of Gammarus showed that this efficient shredder exposed in an circumneutral stream did not feed on leaves conditioned in acidic water, indicating that the quality of food may represent an important factor in acidified ecosystems. One the same way, Gammarus exposed in an acidified stream did not feed on leaves whatever the type of conditioning.

INTRODUCTION
To date, acidification of running waters in France has been studied mainly in the Vosges Mountains (Massabau et al., 1987; Probst et al., 1990; Guérol et al., 1993; Guérol et al., 1995; Party et al., 1995). A recent physico-chemical survey performed over 392 headwater streams draining forested catchments lying on granite or sandstone, has demonstrated that during low-flow, more than 60% of the streams had a ANC ≤ 50 μeq L⁻¹ (Guérol et al.,
1997). Consequently, acidification must be considered as a major cause of headwater stream perturbation in the Vosges Mountains.

Biological assessment of freshwater ecosystems usually consists of a limited number of indices and parameters such as richness, abundance, diversity, taxa distribution of macroinvertebrates, algae or fish. Such approaches ignore system-level responses and may not be reflective of overall ecological health. A better alternative may be to assess stream health by the use of an array of parameters obtained from different organisational levels. Such multimetric studies integrate information from individual, population, community and ecosystem levels (Barbour et al. 1995) and minimise weaknesses that metrics may have individually. In order to survey and to assess the evolution of running water ecosystems, different simple, efficient and low-cost biological methods were tested in headwater streams draining areas of the Vosges Mountains subjected to acidification.

MATERIALS AND METHODS

As shown by Fig. 1 different biological parameters were studied at 3 organisational levels of the ecosystem.

![Synthetic schema showing the different types of parameters studied and the corresponding organisational level.](image-url)
**Study area**

Studies were performed in the Vosges Mountains, an old massif located North-eastern of France, close to the German border (Fig. 2). All sampling sites are located upstream of any anthropogenic activities (no housing, no farming and no industries). It is important to mention that due to local differences in the elemental bedrock composition it is possible to find in a same catchment both circum-neutral and acidified streams.

**Macroinvertebrate communities**

Macroinvertebrate communities of 41 streams were sampled during late spring and autumn by using a Surber sampler (total sampled surface : 0.26 m²; mesh aperture 350 µm). Invertebrates were determined to the lowest practical taxonomic level (mainly species or genus for Plecoptera, Ephemeroptera, Trichoptera, Coleoptera, Crustaceans, and Mollusces ; to genus or family for Diptera).

The biotic index of acidification (B.I.A.) is a simple biological method we have proposed to the Agence de l’Eau Rhin-Meuse (a regional French Agency for Water management) to assess acidification in the Vosges mountains (Guérol et al., 1991; 1997). This method is based both on the taxonomic richness and on the acid-sensitivity/tolerance of macroinvertebrates in the Vosges Mountains. The value of the index varies from 0 (typical stream in the Vosges Mountains with reference community) to 9 (very acidified stream with residual community) (see annexe).

**Trout blood analyses**

*Transferred trout*: two-year old brown trout (*Salmo trutta fario*) obtained from a local fish farm were exposed in 3 streams chosen in order to include an acidified stream (Rouge-Rupt: mean pH: 4.9; mean total Al: 203 µg L⁻¹; mean Ca: 42 µEq L⁻¹), a slightly acidified (Grands-Clos: mean pH: 6.1; mean total Al: 80 µg L⁻¹; mean Ca: 71 µEq L⁻¹) stream and a circumneutral stream (Tihay: mean pH: 6.8; mean total Al: 27 µg L⁻¹; mean Ca: 166 µEq L⁻¹). Six trout were randomly collected in each stream after 48 and 96 hours of exposure.

*Autochthonous trout*: autochthonous brown trout were collected by electrofishing in 26 steams characterised by different levels of acidification. Note that among these streams, some were annually restocked with trout eggs during the last 10 years.

Samples of arterial blood were taken from living fishes by a puncture in the caudal artery and collected into heparinized tubes. To measure plasma parameters, blood samples were centrifuged for 5 min. at 8000 t / mm. Cl⁻ and Na⁺ concentrations were determined by using ion chromatography and atomic absorption spectrophotometry respectively.

**Leaf litter breakdown**

The impact of acidification on coarse allochthonous material breakdown was performed using plastic baskets filled with common beech leaves (*Fagus sylvatica*) and placed in 7 streams.
showing different degrees of acidification. One stream was highly acidic (pH=4.6; Al tot = 801 µg.l⁻¹), 3 were acidic (pH = 4.95 to 5.2; 250 < Al tot <300 µg.l⁻¹), 1 was slightly acidic (pH = 6.06; Al =144 µg.l⁻¹) and 2 were circum-neutral (pH = 6.64 to 7.4; Al<100 µg.l⁻¹), typical of healthy Voosian streams. Two types of baskets (5 mm and 0.3 mm mesh aperture) were used in order to evaluate the part of the mass loss due to macroinvertebrate shredding activity. The remaining dry mass was measured after 10 days (loss due to leaching) and 196 days. Macroinvertebrates which had colonised the baskets were sorted and identified to the lowest practicable taxonomic level.

**Feeding activity of Gammarus fossarum**

The experiment was conducted in April 1998, in the same streams used for experiment with transferred trout (see experiment with transferred trout). Beech leaves (*Fagus sylvatica*) collected after abscission were conditioned during 136 days in an highly acidic stream (pH: 4.68, alkalinity: -19 µEq.l⁻¹, total Al: 801 µg.l⁻¹) (further mentioned as CAS leaves) and in a circumneutral stream (pH: 7.36, alkalinity: 539 µEq.l⁻¹, total Al: 36 µg.l⁻¹) (further mentioned CCS leaves).

Adult males of *Gammarus fossarum* were collected from the circumneutral stream (see above). For each diet tested (CAS and CCS leaves) 3 groups of 10 individuals were maintained in cylindrical pierced pots covered with a net. Feeding trial units were then placed in tanks anchored on the bottom of the streams. In each tank, the 3×2 groups of 10 gammarids were each provide with five 2-cm diameter disks of the two types of beech leaves. After 6 days, organisms were removed from the streams. Gammarids and leaf disks were dried at 60°C for 2 days and weighted. Leaf disks were then scanned and computed. For each group, total remaining area of the disk leave was calculated using a Soft Imaging System (AnalysYS 2.11, OLYMPUS). Remaining area and final dry mass results were used in order to calculate leaf remaining mass in each pot. By difference, we obtained consumed leaf mass. Feeding activities were expressed as mg dried leaves/g of dried *G. fossarum*.

**Chemical parameters**

Chemical analyses were performed at different occasions depending on the type of study. Water samples were filtered in the field with pre-rinsed cellulose nitrate Sartorius filters, 0.45 µm pore diameter, and were analysed within a period of 3 days after collection. pH was measured in the field using a combined glass electrode compensated for temperature. The determination of chemical composition was performed by ion chromatography (inorganic anions), flame absorption spectrophotometry (K, Na) and inductively coupled plasma emission spectrometry (ICP-AES) (total Al, Ca, Fe, Mg, Mn, Si). Organic carbon was determined with a Carlo Erba analyser. Al speciation was obtained according to a recent improved procedure (Boudot et al., 1994). Alkalinity was determined by Gran’s titration.
RESULTS

Macroinvertebrate community

**Taxa richness.** Fig. 3 gives the highly significant relationship we observed between the taxonomic richness and the mean pH of 41 streams. The taxonomic richness severely decreased with decreasing pH. In the most acidified streams, the taxonomic richness was more than 50% depleted compared to circumneutral streams. The most common and acid-sensitive taxa were *Ancylius fluviatilis* (Mollusc), *Gammarus fossarum* (Crustacean), *Epeorus sylvicola*, *Ecdyonurus venosus*, *Rhithrogena gr. semicolorata* (Ephemeroptera), *Dinocras cephalotes*, *Ferla marginata* (Plecoptera), *Liponeura cinerascens* (Diptera).

Sensitive taxa were represented by *Glossosoma conformis*, *Hydropsyche pellucidula*, *Philopotamus montanus* (Trichoptera), *Baetis alpinus*, *B. melanonyx*, *B. muticus* (Trichoptera), *Ibisa marginata*, *Berdienella sp.* (Diptera), *Hydraena gracilis* (Coleoptera) whereas acid-tolerant taxa were *Protonemura junosa*, *P. nitida*, *Brachyptera seticornis*, *Leuctra nigra*, *L. hypopus*, *L. inermis*, *L. cingulata*, *Nemoura chionea*, *Amphinemura sulciocollis* (Plecoptera), *Rhacyphila sp.*, *Plectrocnemia conspersa*, *P. geniculata*, *Drusus annulatus* (Trichoptera), *Chironomidae*, *Limoniidae*, *Empididae* (Diptera), *Oreodytes sp.* (Coleoptera).

It is important to note that in some streams the macroinvertebrate richness began to decrease from a mean pH value around 6.5

**Biotic Index of Acidification (B.I.A.).** Fig. 4 gives the relationship between the Biotic Index of Acidification (B.I.A.) and the mean pH. We observed a highly significant dose-response relationship between the BIA and the mean pH. The lowest value (0), meaning that a stream is not affected by acidification, was obtained only for streams with a mean pH above 6.5 whereas streams with a mean pH below 5.0 showed the highest value (9) meaning that these streams were severely acidified and had residual communities.

A same dose-response relationship was observed between the B.I.A. and the mean ANC (Fig. 5). Some streams began to exhibit impoverished communities for a mean ANC values ranging from 50 to 100 μeq L⁻¹. However severe loss of taxa occurred only under a mean ANC value of 50 μeq L⁻¹. Once the acid-sensitivity of taxa in a given area is determined, such a simple method may constitute a useful tool to monitor acidification.

**Trout blood Na and Cl concentrations**

Plasma Na and Cl concentrations in transferred and autochthonous trout are illustrated by Fig. 6 and 7.

**Transferred trout.** After 48 hours of exposure, trout transferred in the acidic stream (pH : 4.9) showed a depletion of plasma Na and Cl (46 and 26 meq L⁻¹ respectively) compared to trout exposed under slightly (pH : 6.1) or circumneutral conditions (pH : 6.8). After 96 hours
Figure 3: Relationship between the taxonomic richness and the mean pH \((n = 41)\). The solid line represents the mean pH value below which the richness began to decrease in some streams.

Figure 4. Relationship between the Biotic index of Acidification and the mean pH. The solid line represents the mean pH value above which streams are not impacted by acidification \((B.I.A. = 0)\).

Figure 5: Relationship between the B.I.A. and the mean ANC of 41 headwater streams
of exposure at pH 4.9, plasma Cl continued to decrease whereas plasma Na remained unchanged.

**Autochthonous trout.** No relationships were found between plasma ion concentrations in autochthonous trout and any chemical parameters. Such an observation demonstrates that acid-tolerant organisms have been naturally selected and are adapted to survive in low mineralised and moderately acidic waters. The occurrence of fish in numerous acidified streams where trout had supposedly disappeared between 1975 and 1985, results from repeated annual restocking with fertilised eggs obtained from local populations of *Salmo trutta fario* occurring naturally in healthy headwater streams.

Evidence of ionregulatory failure has been demonstrated from a long time in many species of fish (Muniz and Leivestad, 1980; Witters, 1986; Gagen and Sharpe, 1987; Booth et al., 1988). Consequently, as previously proposed by Roche and Boge (1996), the use of blood parameter (such as plasma Na and Cl concentrations) in transferred fish represents a simple and efficient method to quickly assess the toxicity of streamwaters.

**Leaf litter breakdown**

In all selected streams, initial leaching was low (mean value = 4.18% ± 0.8) and did not appear to be influenced by water quality (Fig. 8). On the opposite results on mass loss of leaves placed in coarse mesh baskets showed that beech leaf breakdown was drastically affected under acidic conditions since after 196 days only 15.8% (±1.5) of the initial mass was lost in the acidic streams vs 83.6% (±2.7) in the circumneutral streams. Note that whatever their degree of acidification, all acidic streams presented similar response in term of leaf breakdown. Slightly acidic stream showed intermediary response with 27.91% of the initial mass lost after 196 days.

Differences between leaf mass loss in coarse mesh baskets (CM) and fine mesh baskets (FM) revealed that macroinvertebrates had a large influence on leaf breakdown under circumneutral conditions (83.6% in CM vs 25.0% in FM) whereas under acidic conditions, their role appeared to be very weak (15.8% ± 1.5 in CM vs 14.8% ± 1.3 in FM).

As previously exposed in this paper, acidification has a drastic impact on macroinvertebrate communities by eradicating numerous acid-sensitive taxa. In this sense, the analysis of macroinvertebrates colonising leaves showed that shredder communities were affected both in terms of abundance and taxonomic composition under acidic conditions. Indeed, in acidic streams (even in the slightly acidic one) *Gammarus fossarum* was not present and was replaced by Plecoptera Nemouroidea (notably Leuctra sp., Nemoura sp., Protonemura sp., Amphinemura sulcitellus). Gammarids in the non-acidic streams and Nemouroidea in the acidic streams presumably had very different impacts on litter fragmentation.

Although further investigations are needed, we suppose that the absence of the acid-sensitive species *G. fossarum* from the acidic streams partly explains the different breakdown rates observed. The disappearance of such a key shredder, due to the toxicity of the acidic water, presumably is of major importance to explain disturbance in acidified stream functioning.
Figure 6: Trout plasma Na⁺ (a) and Cl⁻ (b) concentrations after 48 and 96 h of exposure in three different streams.

Figure 7: Plasma Na⁺ and Cl⁻ in autochthonous trout living in 26 streams characterised by different levels of acidification.
Feeding activity of *Gammarus fossarum*.

The most striking result was that *Gammarus fossarum* almost completely stopped its feeding activity in the acidic stream for both individuals fed with CAS and CCS leaves, when compared with the reference stream (Fig. 9). After 6 days of exposure, only $3.5 \pm 0.8$ vs $395 \pm 171$ mg dry CCS leaves/g dry gammarids and $3.0 \pm 0.2$ vs $139 \pm 14$ mg dry CCA leaves/g dry gammarids were consumed. This means that feeding activities on both CAS and CCS leaves were 113 and 46 times lower in the acidic stream than in the reference stream, respectively.

In the slightly acidic stream, no significant differences in gammarid feeding activity on CCS leaves were observed when compared to the reference stream. However, gammarids fed on CAS leaves showed significant reduction in their feeding activity ($20 \pm 19$ vs $139 \pm 14$ mg CAS leaves/g gammarids, in both slightly acidic and reference stream, respectively) which was comparable to the feeding activity of gammarids exposed in the acidic stream. Our findings show that not only the quality of the water but also the quality of the food may have an impact on the feeding activity of gammarids.

CONCLUSION

In the Vosges mountains, chemical comparisons of stream waters between 1991 and 1997 tend to show a global decrease of calcium leading to concentrations which could become more and more critical for the survival of acid-sensitive species (in circumneutral streams) as well as for acid-tolerant organisms (in acidified streams). Consequently, it appears necessary to assess the actual evolution of ecosystems. In this sense, the multi-parameter study we have performed appears interesting as it allows to assess the health state of individuals, communities as well of the ecosystem. For example, the loss of species such as the efficient shredder *Gammarus fossarum* may have a great impact on basic ecological processes such as the breakdown of allochthonous material.
Figure 8: Leaf mass loss after 196 days of exposure in streams characterised by different levels of acidification.

Figure 9: Leaf consumption by *Gammarus fossarum* exposed *in situ* during 6 days under different acidic conditions.
References


Booth C.E., McDonald D.G., Simons B.P., Wood C.M. 1988. Effects of aluminum and low pH on net ion fluxes and ion balance in the brook trout


Annexe: DETERMINATION OF THE BIOTIC INDEX OF ACIDIFICATION (B.I.A.)
7. Invertebrate communities of high mountains lakes (Tatra Mountains) as acid pollution indicator*

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*This is an extended abstract, the full paper will be published later

High concentrations of sulphur and nitrogen in industrial emissions have resulted in large areas of the world being endangered by acid rain. In regions with calciferous or other bedrock with high buffering capacity, even substantial amount of acid rain do not adversely affect water chemistry. On the contrary, lakes situated on granite bedrock and associated soils with little or no buffering capacity e.g. lakes in Scandinavia and High Tatra Mountains are highly acid-sensitive and both their chemistry and biology respond rapidly to changes in acidic inputs. In recent years there have been many studies in such lakes of water chemistry changes brought about by acid rain pressure. Several studies describing this problem have been carried out in the Polish Tatra Mountains Region (Wojtan and Galas 1994, Bombowma and Wojtan 1996, Galas et al. 1996, RyczkoD and Worsztynowicz 1995). Hydrochemical and hydrobiological study on four Tatra lakes have been carried out to address the manner in which acidification processes influence the Tatra lakes bioecenos.

The investigations were carried out in four high mountain lakes situated above the timber line in the area of Tatra National Park (Figure 1). In two lakes Zielony Staw and DBugi Staw lakes the period of ice cover lasts 5-8 months. Both lakes are filled with water throughout the year, however, the surface water level decreases significantly in DBugi Staw lake in winter, even to 5 m, but it never dries out. In Dwoisty Staw lakes during the winter time water disappears through the underground outflow and in spring these lakes fill up with water from melting snow. The glacial relict, Branchinecta paludosa (Crustacea) inhabited those lakes till sixties (Dyduch-Falniowska and Smagowicz 1980). The substratum in each lake is different. The middle part of Dlugi Staw lake bottom is covered by moss Warnstorffia exannulata (1 m, height), the presence of which was detected at the beginning of this century (Olszewski 1948). There are also big boulders, stones and some mud. The Zielony Staw lake bottom is covered by mud, while bottom of both Dwoisty Wschodni and Zachodni lakes are covered entirely by big boulders. Fish, introduced Salvelinus fontinalis, are present only in Zielony Staw lake and they have significantly changed the zooplankton composition (Gliwicz 1985) and probably also influenced benthic invertebrates.

Because of the predominantly muddy substrate in Dlugi Staw and Zielony Staw lakes quantitative samples of benthic invertebrate were taken using corer while qualitative samples from stony littoral areas of these lakes were taken using the kick method. In Dwoisty Zachodni and Wschodni lakes qualitative benthic samples were taken by SCUBA-diver. The comparison of the percentage composition of fauna species was made.

The studied lakes are situated very close to each other, in the same valley. They have ultraoligotrophic waters with very low conductivity (9.3-18.7 μS). It increased during the snow melting period (43 μS in Dlugi, 35 μS in Dwoisty Wschodni lake and 45 μS in Dwoisty Zachodni lake). The lakes have low calcium concentration (average 2.5 mg Ca dm⁻³) and magnesium (average 0.6 mg Mg dm⁻³). With the exception of Zielony Staw, the waters of these lakes are acidic; the lowest pH values (4.2 - 4.5) are noted just after the snow melting period; however, later the pH slowly rises and in summer the maximum pH value in Dwoisty Staw lake is 6.2 - 6.5 while in DBugi Staw lake the corresponding value is 5.8 only. In earlier studies from 1956-1964 years (Oleksynowa and Komornicki 1989) on those lakes similar values of pH in summer time were found.
Figure 1. The investigations were carried out in high mountain lakes situated in the Tatra National Park.

In all studied lakes Nematoda, Oligochaeta and Chironomidae were dominant. The percentage share of the remaining groups was less than 10%. Nematodes were very abundant, except for the stony littoral areas of Długie and Zielony Staw lakes. A high number of Chironomidae was observed in most of the studied lakes and habitats, however, they were less abundant in the littoral zone of Długie Staw lake and in the mud of Zielony Staw lake. Percentage share of Oligochaetes varied from lake to lake and it is difficult to explain the large variation in their distribution. They were most abundant in Długie Staw
lake littoral (89%) and in Zielony Staw lake profundal (68%). In Dwoisty Stawy lakes they were less abundant (3.8%; 11%), possibly due to the freezing out cocoons during wintertime.

In the studied lakes 51 taxa were found in total, the highest number was in Lake Długi Staw (29) and Zielony Staw (28), lesser numbers of taxa were recorded in Dwoisty Wschodni (23) and Zachodni (21) lakes. The greater faunal diversity recorded in Zielony Staw lake might result from a higher trophy status; all 4 lakes have been described above as ultraoligotrophic while in Długi Staw the investigation was of four years duration compared with two years in the Dwoisty Stawy lakes. In the last two lakes (Długi Staw and Dwoisty Staw) drying and freezing to the bottom may also cause the lower number of species found.

The dominant species in the profundal zone of Lake Długi Staw were Micropsectra coracina and Heterotrissocladius marcidus (Chironomidae), and Cernosvitioviella tatrensis (Oligochaeta). In the littoral zone only Oligochaeta dominated: Cognettia spp. (amphibiotic genus), while Chironomidae abundance was much more lower. In both Dwoisty Staw lakes Chironomidae dominated: in Wschodni lake Zavrelmiya sp., Crictopus (C.) gr. fuscus and Corynoneura sp in smaller number. In Zachodni lake the most dominant larvae was Corynoneura sp., next was Zavrelmiya sp. and Macropelopia sp. A further important element of fauna in both Dwoisty lakes was Oligochaeta - Cernosvitioviella tatrensis. In the profundal zone of Zielony Staw lake Nits variabilis (Oligochaeta) dominated and while in the littoral stony zone Chironomidae: Heterotrissocladius marcidus, Corynoneura sp. were prominent. The character of the Oligochaetes' fauna of muddy bottom of this lake indicates its higher trophy when compared with the remaining studied lakes.

In the studied lakes, in spite of some differences, the composition of benthic invertebrates (mostly Chironomidae) was characteristic of oligotrophic high mountain lakes as was stated already in 30-ties (Zav'fel 1935, Hrabe 1942, Thienemann 1954). Also Cernosvitioviella tatrensis was very common and constantly (or permanently) observed in Tatras' lakes since the beginning of the century (Kowalewski 1917, Hrabe 1942) until now. Biocenoses living in studied lakes are adapted to periodical changes of pH values. There were no taxa characteristic for strongly acidified waters e.g. Zulotschia taurica (Chironomidae), which presence might provide a proof that process of acidification increased recently. There was no apparent biodiversity decline which might indicate important ecosystems changes. Explanation of disappearance of Branchiacta paludosa, the relic of glacial epoch, from both Dwoisty Staw lakes by increased acidification (Dyduch-Falniowska 1992) is very unconvincing. This species lives on far North, in acidified periodical lakes and also in Wyżny Furkowny lake (Slovak Tatra Mts) where amount of acid rains is similar to the Polish part of Tatra.

The attempt of explanation of acidification effect on high mountain lakes based only on the water chemistry changes is unsatisfactory. The data from flora and fauna communities should be also included as it was made in studies on Scandinavian lakes. This process should be continuously monitored, especially in lakes with very low trophy, otherwise the mechanism of self regulations of lake ecosystems may fail and further changes will be irreversible.

Acknowledgement
This work was supported by Commission of the European Communities (No EV5V-CT92-0205 and No ENV4-CT95-0007) and by the Polish State Committee for Scientific Research (No 6 PO4F 053 08).
References


8. Hydrobiological Monitoring in Latvia under the ICP Waters and ICP IM Programmes

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Latvia has a wealth of experience in water monitoring. First hydrochemical observations date back to 1946. The existing observational networks generally fall into 2 categories:

- locality that serve to solve internal nature conservation issues
- areas that are representative of regions and are involved in the implementation of international programmes

The regional network provides for:

- the studies of long-range changes in quality of waters exposed to atmospheric pollution
- the investigation into effects of water pollutants on the various compartments of the ecosystem
- the assessment of changes in water under the effects of atmospheric air, precipitation, climate, etc.
- the elaboration of a common approach to environmental monitoring

Hydrobiological investigations have been performed both at the local network and the regional network since 1976.

The local network of 25 observational sites covers areas in the main rivers and lakes under direct anthropogenic impact.

The regional hydrobiological observations are carried out under the ICP Waters and ICP IM programmes under the Convention on Long-range Transboundary Air Pollution, Geneva, 1979. Latvia joined the Convention in 1997.

The programme of the hydrobiological measurements is presented in Table 1. Figure 1 shows the locations of the hydrobiological monitoring sites.
Figure 1. Water quality network in Latvia.
Table 1. The programme of the hydrobiological measurements in Latvia.

<table>
<thead>
<tr>
<th>Programme</th>
<th>Rivers streams</th>
<th>Hydrobiological parameter</th>
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<tbody>
<tr>
<td>Local observation</td>
<td>25 stations on the main</td>
<td>macrozoobenthos: abundance, species diversity, species composition of major groups</td>
</tr>
<tr>
<td>network</td>
<td>rivers and lakes</td>
<td>phytoplankton: total biomass, abundance, biomass of major groups, chlorophyll, pigments</td>
</tr>
<tr>
<td>ICP Waters</td>
<td>L. Jugla, Tulija, Barta</td>
<td>macrozoobenthos: abundance, species diversity, species composition of major groups</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phytoplankton: total biomass, abundance, biomass of major groups, chlorophyll, pigments</td>
</tr>
<tr>
<td>ICP IM</td>
<td>Taurene, Rucava</td>
<td>Phytoplankton: chlorophyll, macroinvertebrates</td>
</tr>
<tr>
<td></td>
<td>(stream and small lake)</td>
<td>Macrozoobenthos: abundance and biomass</td>
</tr>
</tbody>
</table>

The results of an intercalibration in 1997, where we participated for the first time, showed good precision of zoobenthos determinations. However, we had some problems because the codes of biological taxa we applied, sometimes did not correspond to the European classification, especially for Hydrocarina and Diptera.

The stations under ICP Waters programme are representative of the various areas of Latvia and can characterize the background level in the main water basins.

Two ICP IM polygons are located in forest tracks in different physico-geographical zones of Latvia, Seaside Lowland (Rucava) and Vidzeme Hill (Taurene).

Some characteristics of the areas are presented in Table 2.

Table 2. Some site data for the stations in Latvia.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ICP Waters</th>
<th>ICP IM</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Jugla</td>
<td>Tulija</td>
</tr>
<tr>
<td>Catchment area, km²</td>
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<td>Precipitation, mm, yr⁻¹</td>
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<td>727</td>
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<td>Main type of bedrock:</td>
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<td>Forest cover, %</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>Wetland/Bogs, %</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Elevation of site, m</td>
<td>2.29</td>
<td>172.99</td>
</tr>
<tr>
<td>Average runoff, l s⁻¹ km⁻²</td>
<td>8.2</td>
<td>9.3</td>
</tr>
</tbody>
</table>
For assessing pollution of water bodies, trophic water properties, and the state of ecology and acidification, different water quality criteria based on the hydrobiological observation results are applied:

**ICP Waters:**
- Acidification index

**ICP IM:**
- H-Shannon - Wiener species diversity index

**Local network:**
- Spb - saprobic index (Pantle-Buck) of community, Sladecheka modification

To make a complex assessment of surface water pollution at the local network, a Water Quality Classification, based on hydrochemical and hydrobiological indices, is used (Table 3).

As the Raddum’s index used in ICP Waters practice is inapplicable to our region of relatively high pH (7-8), we have used the Shannon-Wiener’s index for the ICP Waters sites.

An attempt was made to calculate the saprobility and Shannon-Wiener indices for a comparative analysis of the state of water bodies exposed to different anthropogenic impacts and with different ecosystem formation conditions (Table 4).

**Table 3. Water quality classification by the hydrochemical and hydrobiological indices.**

<table>
<thead>
<tr>
<th>Class</th>
<th>Characteristics</th>
<th>Saprobility</th>
<th>Inland</th>
<th>Sea</th>
<th>Microbiological index</th>
<th>Microbiological index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>WPI</td>
<td>phytoplankton, zoobenthos</td>
<td>Pantele’s and Bukka’s saprobility index</td>
<td>Concentration of saprophytic bacteria, thousands cells/ml</td>
</tr>
<tr>
<td>I</td>
<td>Very pure</td>
<td>xeno</td>
<td>&lt;0.3</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>II</td>
<td>Pure</td>
<td>oligo</td>
<td>0.3-1.0</td>
<td>0.5-1.5</td>
<td>0.5-5.0</td>
<td>0.3-0.8</td>
</tr>
<tr>
<td>III</td>
<td>Moderately polluted</td>
<td>beta</td>
<td>1.0-2.5</td>
<td>1.5-2.5</td>
<td>5.1-10.0</td>
<td>0.8-1.3</td>
</tr>
<tr>
<td>IV</td>
<td>Polluted</td>
<td>alfa</td>
<td>2.5-4.0</td>
<td>2.5-3.5</td>
<td>10.1-50.0</td>
<td>1.3-1.8</td>
</tr>
<tr>
<td>V</td>
<td>Impure</td>
<td>poly</td>
<td>4.0-6.0</td>
<td>3.5-4.0</td>
<td>50.1-100.0</td>
<td>1.8-3.0</td>
</tr>
<tr>
<td>VI</td>
<td>Heavy impure</td>
<td></td>
<td>6.0-10.0</td>
<td>&gt;4.0</td>
<td>&gt;100.0</td>
<td>3.0-5.0</td>
</tr>
<tr>
<td>VII</td>
<td>Contaminated</td>
<td>&gt;10.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:**
* - Baltic Sea off-shore zone near Ventspils and Liepaja
** - or a random natural hydrocarbon-oxidizing microbial form
Figure 2. Macro-invertebrates in Latvian rivers.
Table 4. The values of the Shannon-Wiener index and the Saprobiity index.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Taurene (ICP IM)</td>
<td>0.81-2.50</td>
<td>2.2-2.27</td>
<td>0.64-3.59</td>
<td>2.05-2.39</td>
<td>0.88-3.42</td>
<td>2.0-2.53</td>
</tr>
<tr>
<td>Rucava (ICP IM)</td>
<td>0.92-2.30</td>
<td>2.13-2.29</td>
<td>0.49-3.42</td>
<td>2.11-2.38</td>
<td>0.91-2.74</td>
<td>1.86-2.64</td>
</tr>
<tr>
<td>Tulija (ICP Waters)</td>
<td>=</td>
<td>=</td>
<td>1.51-2.13</td>
<td>2.08-2.18</td>
<td>1.95-2.50</td>
<td>2.2-2.40</td>
</tr>
<tr>
<td>Barta (ICP Waters)</td>
<td>4.27</td>
<td>2.0</td>
<td>2.67-4.04</td>
<td>2.01-2.30</td>
<td>3.29-3.96</td>
<td>1.94-1.99</td>
</tr>
<tr>
<td>Zaki (ICP Waters)</td>
<td>0.51</td>
<td>2.0</td>
<td>0.37-2.49</td>
<td>1.98-2.09</td>
<td>0.88-2.42</td>
<td>1.55-1.82</td>
</tr>
<tr>
<td>Local network (Lielupe-Jelgava)</td>
<td>2.99-3.66</td>
<td>2.43-2.61</td>
<td>2.81-3.28</td>
<td>2.29-2.48</td>
<td>3.14-3.62</td>
<td>2.32-2.72</td>
</tr>
</tbody>
</table>

The benthos of the water bodies showed a relatively high abundance and diversity of species typical for the Latvian basins in general. The Benthic communities within the areas monitored occur mosaically, depending on the grounds, current velocity and other physico-geographical characteristics, and on the anthropogenic impact.

Saprobiity is a more stable index that reflects mostly the anthropogenic impact on the environment in the same values as the diversity index. For instance, in approximately the same Shannon-Wiener indexes for the river Barta and Lielupe - Jelgava, the saprobiity index is higher for Lielupe exposed to intensive anthropogenic load (Figure 2).

The makrozoobenthos values have not varied significantly in the water bodies during the last years. According to the saprobiity index, the sites of the local network are considered α-β mesosaprobic, while ICP Waters and ICP IM sites are oligo-β-mesosaprobic.

Conclusions and proposals

1. The Shannon-Wiener index and saprobiity index may be used for the assessment of biotas in different water sections.

2. It is evident that the phytoplankton observation results from the ICP Waters sites should be used more actively and annually reported to NIVA, together with the zoobenthos data and the hydrochemical indices, by analogy with the ICP IM programme.

3. The intercalibration results show that it is necessary to have the codes of biological taxa especially for Hydracarina, Diptera and the updated ones for other groups.

4. To continue to practise carrying out zoobenthos intercalibrations and to arrange jointly with the ICP IM phytoplankton intercalibrations.

Ian C. Simpson, Environmental Change Network, UK

Introduction

NoLIMITS is a two year (March 1998 - February 2000) Preparatory Action funded by the European Network for Research in Global Change (ENRICH). The aim of the Action is to develop a strategy for the creation of a pan-European Long-term Integrated Monitoring Site Network. The network, based on existing sites, will be designed to serve the data and information needs of European-scale users for the detection and interpretation of global environmental change in terrestrial and freshwater systems. To succeed, the network must be able to deliver policy relevant information that either cannot be provided by alternative mechanisms or is better and more cost effective than information available through existing channels. To populate the network with the required data and information, benefits of participation must also be clearly evident to contributing sites e.g. through enhanced research opportunities, publicity and acclaim.

NoLIMITS Vision

"To create a European network of sites for long-term integrated monitoring which addresses local, national, European and global scale requirements for policy relevant data and information and provides a focus for scientific collaboration related to research on environmental change and its consequences."

Rationale: The broad scale detection of environmental change arising from long-term monitoring programmes has been of proven value in warning politicians and the public about the dangers of damage to the environment and in informing policy responses. As a result there is now increased awareness, particularly amongst users of environmental data and information, of the role that coordinated measurements across broad networks may have in detecting, interpreting and reporting on global change. In practice, potential users are unable to make effective use of existing resources because of the lack of common approaches to data collection, management and presentation. Improved networking on a pan-European scale would provide for more cost effective use of long-term data and a more powerful data and information source to enable the earlier detection of environmental change.

Although there are a large number of sites across Europe which already undertake some form of long-term integrated monitoring, there are only a few national and international initiatives aimed at the development of integrated monitoring networks. The Global Terrestrial Observing System (GTOS) Database TEMS (Terrestrial Ecosystem Monitoring Sites) lists contacts for nearly 500 monitoring sites in Europe. Some of these are already part of national networks (e.g. the UK Environmental Change Network (ECN)), some are part of international programmes which address specific environmental issues (e.g. ICP IM - the International Cooperative Programme on Integrated Monitoring on Air Pollution Effects) or particular habitats or biomes (e.g. European Forest Network), but many are still isolated sites, particularly in eastern Europe.

Three features distinguish NoLIMITS from related activities: 1) development plans will be driven by defined user, rather than research, requirements; 2) the emphasis on "integrated" monitoring of the pan-European terrestrial environment; and 3) an Internet-based mechanism for information management and delivery. Special emphasis will be placed on the development of models and analytical tools to process data into readily understood policy relevant information and indicators.
NoLIMITS Objectives

- To explore costs and benefits of, and make recommendations for, establishing a European long-term integrated monitoring site network, based on existing terrestrial sites;
- To identify European-scale user requirements for data and information from such a network;
- To identify immediate actions to improve the coherence of existing sites, particularly in relation to emerging opportunities for integrated monitoring schemes in Eastern Europe;
- To identify priority areas for research and development which will enhance the development of the network to meet the specified user requirements; and
- To establish a self-sustaining information exchange network harnessing the capability of the Internet, to provide a forum for the exchange of information between sites and users of the data and information.

The NoLIMITS Partners are: Environmental Change Network (ECN), International Monitoring Programme (ICP IM), The Hungarian Academy of Science Institute of Ecology and Botany, European Environment Agency (EEA), Global Terrestrial Observing System (GTOS), Centre for Earth Observation (CEO).

User Requirements for European scale integrated monitoring

NoLIMITS user requirements will be defined in terms of measurements, information requirements and delivery mechanisms for specific users (EEA, GTOS, CEO) and general user groups (in policy, research, commerce, education). The mechanism to identify these requirements is currently under development but will include consultation with key users and constitute a central theme for the NoLIMITS International Workshop (24-26 March 1999). The difficulty of defining user requirements should not be underestimated as the NoLIMITS concept falls foul of the “chicken and egg” syndrome. Without knowing precisely what the user requirements are it is difficult to define the required network and without knowing what the network will look like it is difficult to define what it will be capable of delivering.

Some of the user requirement questions NoLIMITS will address:
- What level of data and information are required?
- What criteria should be used for site selection?
- Should all sites be required to measure all variables?
- Are common protocols and data standards necessary?
- What are the most important groups of variables that need to be integrated?
- How should data and information be collated, integrated and delivered?
- How should the network be coordinated?

NoLIMITS Modus operandi

(1) Task Force with representatives from each project partners - to prioritise, initiate and coordinate activities, and liaise with: national contacts, users, and ENRICH.
(2) International Workshop (c 80 -100 invitees) Brasenose College, Oxford, 26-29 March 1999. The workshop will: (i) develop a rationale for a network of long-term integrated monitoring sites across Europe, based on user requirements; (ii) bring together potential users and providers of data and information from across Europe; (iii) develop a strategy for the development of a European network; (iv) identify research opportunities arising from closer links between sites across Europe; and (v) identify the next steps required to facilitate the development of a European network of sites.
(3) There will be at least two Working Groups (10-20 participants) to identify research and development priorities. A working group looking at modelling issues is currently being set up, other working groups may be established to examine some of the following issues in more detail: (i) Network definition (design
and implementation; (ii) the information exchange network; (iii) integrating socio-economics and environmental site monitoring; (iv) defining user requirements; (v) links with sectoral, national and international networks; (vi) presenting biodiversity data from terrestrial ecosystems as comparable indicators.

(4) An Information Exchange Network will be developed to promote the exchange of information between sites, and between sites and end-users. NoLIMITS will help to develop standard approaches to the presentation of metadata and summary information (indicators) and will encourage sites to evolve towards mutually consistent programmes of measurements. The Information Exchange Network will be self-sustaining and provide a focus for the networking of European long-term monitoring sites. This Network is likely to be based on a dispersed system with a focal point on a central server (probably with regional nodes) which provides links with other sites in the Network. The central server will probably host a searchable metadatabase for sites that contribute to the network. Individual sites (or groups of sites) will set-up and maintain their own pages which might include: site descriptions; high level metadata on data collections; summary information and/or indicators; research initiatives; and links to other NoLIMITS participants. A Pilot Study is underway to develop a prototype system to be demonstrated at the International Workshop.

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10. An Appraisal of Biological and Physico-Chemical Assessments of Water Quality

Jim Bowman, Environmental Protection Agency, Ireland

Introduction

In Ireland the principal forms of aquatic pollution encountered are eutrophication and organic pollution; less frequently noted are toxic pollution and acidification of sensitive areas. These and all other forms of pollution cause changes in the physico-chemical and biological characteristics of the receiving waters. Thus, water quality and the extent of pollution can be assessed by monitoring the changes to the chemistry and the biology of the waterbodies. In practice, a combination of both approaches is preferable to either on its own; although, in Ireland the overall assessment of river and lake water quality is based largely on the results of examination of the aquatic flora and fauna.

Increasing emphasis is now being placed on the broader ecological quality of the aquatic environment and in Europe the soon to be introduced Framework Directive will have as its objective the maintenance, or where necessary the restoration, of ecological damage to ensure, in so far as is possible, good aquatic ecosystems. It will no longer be sufficient to show that the chemical composition/quality of the waterbody is adequate to satisfy the requirements of the most sensitive aquatic organisms; instead, it will also have to be demonstrated that sustainable populations of these organisms are present in the waterbody. In order to achieve this end, suites of chemical and biological parameters are included in a comprehensive monitoring schedule.

Biological Assessment of Water Quality

In rivers the macroinvertebrate communities are recognised as containing organisms most sensitive to the stresses caused directly or indirectly by various pollutants. The response and sensitivity of these organisms to pollution has been well studied and documented over the last century and their use as pollution indicators in the context of water quality assessment is widespread. The macroinvertebrate communities have limited ability to avoid pollution incidences by drifting or swimming to cleaner areas and thus are exposed to and reflect the water quality conditions at the sampling point. The basis for water quality assessments based on these organisms is the degree to which the faunal communities, at a sampling site, deviate from the expected or previously measured unimpaired populations.

In order to express measured changes in macroinvertebrate communities in a simple intelligible form several numerical scales or indices are employed. To describe the changes due to organic pollution in rivers many countries have developed scales or indices that best suit conditions within their regions. However, in the case of acidification one scheme, the Raddum Index, has gained wide acceptance as an accurate means of expressing the changes to the macroinvertebrate communities as a result of increased inputs of artificial acidity.

In Ireland other aspects of the biota, such as benthic algae and macrophytes, are also considered in the general quality assessments. Abiotic factors such as current speed, water turbidity and depth, sillation and substratum type as well as oxygen saturation and temperature at time of sampling are also taken into account in the assessment procedure. These are factors which also have a bearing on the abundance and diversity of the macroinvertebrate communities.

In lakes the extent of planktonic algal and cyanobacterial development, measured in terms of the green algal pigment chlorophyll, is widely recognised as a good indicator of water quality where eutrophication
is the dominant form of pollution. However, this approach is less successful in assessing the level of acidification and other forms of pollution and macroinvertebrates communities from littoral areas are considered to be the better indicators.

**Physico-Chemical Assessment of Water Quality**

Discrete samples taken at frequencies which vary widely characterise the majority of physico-chemical monitoring programmes. While biological methods of assessment give an indication of the water quality conditions at the sampling point for a period, maybe as long as several months, prior to the time of sampling the physico-chemical analysis of parameters on a discrete water sample strictly relate to the conditions at the time of sampling. Nevertheless, while such a sample may not appear to be spatially and temporally representative of the waterbody, it has been shown that, in the absence of exceptional hydrometric and effluent discharges, discrete samples can give a reliable description of the chemical composition of the water. However, in circumstances where these discharges are irregular the reliability of such a sample may not be high and the interpretation of data arising from such a sample requires care.

The parameters measured in a physico-chemical monitoring programme will reflect the type of pollution likely to be present; however, with the increasing complexity of industrial effluents this may prove to be difficult. The assessment of the quality is based on a comparison of the physico-chemical measurements made with known threshold levels or standards set out in national and international legislation.

**Comparison of Aspects of Physico-chemical and Biological Water Quality Assessment Techniques**

Set out in Table 1 is a comparison of the two assessment techniques. Physico-chemical measurements identify and precisely quantify the chemical composition of the sampled water and the concentration of pollutants, if any, therein but can not indicate if they are having an impact on the biota. An examination of the biological communities, on the other hand, can highlight any impacts the pollutants may be having on the biota. However, a biological assessment can not identify chemical components in the water or indicate the cause of any ecological change with any degree of confidence.

Biological water quality assessment techniques are successful when the stresses exerted by a particular combination of pollutants lie within the range of sensitivity of the specific organisms or communities being examined. When the concentration of pollutants exceed this range resulting in the demise of the organisms there is no further biological response to the increased pollution and the assessment technique is of limited use. In these extreme circumstances physico-chemical assessment techniques only are reliable.
Table 1. Comparison of features of Chemical and Biological Water Quality Assessment Techniques.

<table>
<thead>
<tr>
<th>Performance</th>
<th>Chemical</th>
<th>Biological</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pollutant concentration assessment</td>
<td>good</td>
<td>poor</td>
</tr>
<tr>
<td>Pollutant identification</td>
<td>good</td>
<td>poor</td>
</tr>
<tr>
<td>Measure of impact</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>Cost</td>
<td>high</td>
<td>low</td>
</tr>
<tr>
<td>Single sample value</td>
<td>poor</td>
<td>good</td>
</tr>
</tbody>
</table>

Where extensive water quality monitoring is necessary, such as on a national or regional scale, and where a large number of unpolluted rivers are to be examined, the physico-chemical monitoring approach is expensive in terms of human and financial resources. To obtain a reliable assessment of water quality conditions in this manner would require sampling at a monthly or higher frequency on watercourses in receipt of waste discharges. In contrast, just two biological samples per annum (winter and summer) would normally provide a reasonably accurate assessment of the water quality; a considerably greater number of physico-chemical samples would be required to achieve such an assessment with the same degree of confidence.

The standards or threshold values against which the measured parameters are considered in the physico-chemical assessment techniques are frequently expressed in terms of annual mean, maxima or percentiles. This commonly means data collection for a duration of at least one years before a reliable assessment of the water quality can be made. There are exceptions of course, such as where the annual maximum concentration being sought is associated with a particular event or season i.e. snow melt or a period of maximum or minimum hydrological discharge. In these latter cases the period of physico-chemical monitoring can be of much shorter duration. However, in general, the use of the physico-chemical approach almost invariably precludes a quick reliable assessment of water quality based on a single sample. On the other hand the use of biological assessment techniques, such as the macroinvertebrate communities, can provide an immediate and reliable assessment of quality. These communities acting as continuous monitors of the water in their environment reflect the impacts of any pollutant events occurring in the previous months. This fact coupled with the relative ease of identification of the macroinvertebrate organism means that a biologist can give a reliable assessment of water quality at the sampling site within minutes of taking the sampling.

It is clear from the foregoing that both physico-chemical and biological water quality assessment techniques have their own particular applications, advantages and disadvantages so that only by a combination of both can the limitations of each be overcome and a thorough understanding of the total situation be gained.
11. Chironomids as indicators of acidification. Report from a pilot project in the Northern Lakes Recovery Study

Godtfred A., Halvorsen, University of Bergen, Norway

Introduction

The Northern Lakes Recovery Study (NLRS) is a joint project between Canada and Norway with the aim of studying the natural recovery of anthropogenically acidified lakes. The study involves the Cooperative Freshwater Ecology Unit (Coopunit), Department of Biology, Laurentian University, Sudbury, the Norwegian Institute for Nature Research (NINA), and Laboratory for Freshwater Ecology and Inland Fisheries (LFI), Department of Zoology, University of Bergen.

The study area is the Killarney Provincial Park, 40-60 km south of Sudbury in Ontario. This area has had a heavy input of atmospheric sulphur from the metal smelters in Sudbury, but also due to emissions from the industry in the United States. However, in the last decades the situation has changed due to a reduction in excess of 80% in the emissions from the Sudbury area, and some of the lakes have started to recover. The background history, the affected area and the starting recovery is described in several articles in Gunn (1995).

LFI at the University of Bergen intend to examine the response of the profundal/sublittoral chironomid community to this natural recovery. This paper reports the results from a pilot project run in 1977, focusing on chironomids as indicators of different levels of acidification.

Study area

Killarney Provincial Park is about 485 km², and is situated at about 46° latitude in Northern Ontario. The geology of the park is dominated by slow-weathering bedrock, providing little buffering against acid precipitation. However, some of the lakes have elements of more easily weathering rocks in their watersheds. This means that in a relatively small area we have numerous lakes of varying pH including naturally acid lakes, lakes acidified by human activities, and lakes that never have acidified. Other human impact in the area is small, with no farming and only very few leisure cottages along the borders of the park. The park itself is classified as a wilderness, with restricted visitor traffic.

For the pilot project we chose 19 lakes in the park and one just outside based on pH, accessibility, and the extent of former research in the lakes. The elevation of the lakes ranged from 185 to 304 m a.s.l. The data from the lakes are based on a survey done in 1995 and 1996 by the Coopunit (Snucins and Gunn 1997). Water samples for chemical analysis were taken during the winter 1996.

The lakes were sampled in May – June, and in September – October 1997. One lake (Patritridge Lake) was not sampled this year. Ten samples per lake were taken with a modified Kajak corer, sieved through a 250 µm mesh and stored in alcohol. The samples were then shipped to Bergen and sorted, using a binocular microscope. The sampling in spring was partly done by personell from LFI, University of Bergen. The remaining sampling were done by personell from the Coopunit, Laurentian University, Sudbury.

All samples, except the fall sample in O.S.A. Lake, were taken below the Secchi depth in the assumed profundal zone, in order to minimize habitat variation.
The pH in the winter 1996 varied from 4.8 to 7.2. Figure 1 shows that the pH had increased since 1980 in all of the lakes that were surveyed at that time, some of them closing up to their estimated pre-industrial pH level.

![Figure 1. Development in pH in the 19 Killarney Lakes from 1980 to 1995-96. * indicates estimated preindustrial pH based on data from diatoms and chrysophytes (Sushil Dixit (Queen's University), cited in Snucins and Gunn 1997).](image)

All lakes are classified as oligotrophic. Total phosphorus varied between not detectable (< 0.002 mg/l) and 0.006 mg/l, and total nitrogen between 0.08 mg/l and 0.32 mg/l. Some physical and chemical characteristics of the lakes are listed in Table 1. The labile aluminium values were estimated using the LaZerte equation (4) (LaZerte 1984) for lakes with dissolved organic carbon (DOC) >1 mg/l. The values for the clear lakes (DOC < 1 mg/l) were estimated based on a multiple regression from earlier studies in 14 Killarney lakes (Ed Snucins, Coopunit). The equation is as follows:

\[
\text{Labile Al} = 1047 - 1383 \times (\log \text{pH}) + 0.433 \times (\text{total Al}) \quad r^2 = 0.75, N = 14
\]

The calculation of the acid neutralising capacity (ANC) of the lakes was done by Atle Hindar and Arne Henriksen, Norwegian Institute for Water Research (NIVA), in connection with a recent study done in the Killarney Provincial Park (Hindar and Henriksen 1998).
Table 1. Some physical and chemical characteristics of the Killarney Lakes. ANC = acid neutralising capacity. DOC = dissolved organic carbon.

<table>
<thead>
<tr>
<th></th>
<th>pH 98</th>
<th>Sample depths 97</th>
<th>Secchi depth</th>
<th>Max depth</th>
<th>DOC (mg/l)</th>
<th>Total Al (μg/l)</th>
<th>Labile Al (μg/l)</th>
<th>ANC (μekV/l)</th>
<th>Ca (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>7.2</td>
<td>24 28</td>
<td>7.9</td>
<td>28.4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>46</td>
<td>8.40</td>
</tr>
<tr>
<td>Teardrop</td>
<td>6.5</td>
<td>15 13</td>
<td>11.5</td>
<td>16.6</td>
<td>1.1</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>1.65</td>
</tr>
<tr>
<td>Iohnael</td>
<td>6.5</td>
<td>19 19</td>
<td>4.5</td>
<td>19.8</td>
<td>3.5</td>
<td>17.9</td>
<td>1</td>
<td>18</td>
<td>2.75</td>
</tr>
<tr>
<td>Kakakia</td>
<td>6.3</td>
<td>13 18</td>
<td>6.5</td>
<td>30.0</td>
<td>2.7</td>
<td>23.3</td>
<td>4</td>
<td>15</td>
<td>2.30</td>
</tr>
<tr>
<td>Helen</td>
<td>6.3</td>
<td>- 25</td>
<td>5.8</td>
<td>41.2</td>
<td>3.7</td>
<td>64</td>
<td>27</td>
<td>18</td>
<td>2.65</td>
</tr>
<tr>
<td>Bell</td>
<td>5.9</td>
<td>15 18</td>
<td>3.8</td>
<td>28.0</td>
<td>4.9</td>
<td>80</td>
<td>33</td>
<td>14</td>
<td>2.25</td>
</tr>
<tr>
<td>Carlyle</td>
<td>5.9</td>
<td>- 14</td>
<td>4.0</td>
<td>14.6</td>
<td>3.7</td>
<td>74</td>
<td>32</td>
<td>13</td>
<td>1.90</td>
</tr>
<tr>
<td>George</td>
<td>5.8</td>
<td>12 11</td>
<td>9.0</td>
<td>39.7</td>
<td>1.7</td>
<td>94</td>
<td>60</td>
<td>12</td>
<td>1.95</td>
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<td>Partridge</td>
<td>5.7</td>
<td>- -</td>
<td>13</td>
<td>16.9</td>
<td>1.8</td>
<td>70</td>
<td>35</td>
<td>13</td>
<td>2.40</td>
</tr>
<tr>
<td>Johnnie</td>
<td>5.6</td>
<td>13 12</td>
<td>5.5</td>
<td>33.5</td>
<td>3.4</td>
<td>120</td>
<td>61</td>
<td>12</td>
<td>1.60</td>
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<tr>
<td>Tyson</td>
<td>5.6</td>
<td>15.5 22</td>
<td>4.7</td>
<td>39.6</td>
<td>4.6</td>
<td>90</td>
<td>46</td>
<td>21</td>
<td>1.75</td>
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<tr>
<td>Lumsden</td>
<td>5.2</td>
<td>13 12</td>
<td>7.2</td>
<td>22</td>
<td>1.5</td>
<td>175</td>
<td>103</td>
<td>7</td>
<td>1.15</td>
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<tr>
<td>Killarney</td>
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<td>- 13</td>
<td>9.6</td>
<td>60.9</td>
<td>1.0</td>
<td>238</td>
<td>147</td>
<td>10</td>
<td>1.60</td>
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<tr>
<td>Norway</td>
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<td>18 17</td>
<td>10.5</td>
<td>33.6</td>
<td>1.7</td>
<td>260</td>
<td>156</td>
<td>11</td>
<td>1.75</td>
</tr>
<tr>
<td>Burke</td>
<td>5.1</td>
<td>15 16</td>
<td>3.8</td>
<td>15.5</td>
<td>1.8</td>
<td>188</td>
<td>168</td>
<td>9</td>
<td>1.40</td>
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<tr>
<td>Acid</td>
<td>5.0</td>
<td>15 12</td>
<td>4.9</td>
<td>29</td>
<td>1.6</td>
<td>205</td>
<td>169</td>
<td>8</td>
<td>1.10</td>
</tr>
<tr>
<td>Clearwater</td>
<td>4.9</td>
<td>- 12</td>
<td>9.5</td>
<td>13.7</td>
<td>1.6</td>
<td>220</td>
<td>136</td>
<td>7</td>
<td>1.10</td>
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<tr>
<td>Ruth-Roy</td>
<td>4.9</td>
<td>14 12</td>
<td>10.5</td>
<td>18</td>
<td>0.5</td>
<td>340</td>
<td>240</td>
<td>7</td>
<td>1.20</td>
</tr>
<tr>
<td>O.S.A.</td>
<td>4.8</td>
<td>31 12</td>
<td>16</td>
<td>39.7</td>
<td>0.4</td>
<td>206</td>
<td>104</td>
<td>11</td>
<td>2.05</td>
</tr>
</tbody>
</table>

Results and discussion

A total of 41 chironomid species/taxa and 2 chaoborid species were found in the Kajak samples during the spring and fall of 1997. Figure 2 gives the pH ranges of the different chironomid species found in more than one lake. Table 2 shows the pH at which chironomids found in one lake only occurred.

*Chaoborus americanus* Johannsen was found in 3 lakes with pH ranging from 4.9 to 5.2, while *Chaoborus punctipennis* (Say) was found in 7 lakes with pH ranging from 4.9 to 5.9.

![Figure 2. pH ranges of the different chironomid species found in the Kajak samples in 1997.](image-url)
Table 2. Chironomids found at only one pH level in the Kajak samples in 1997.

<table>
<thead>
<tr>
<th>Species</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cricotopus (L) sp. reversus gp</td>
<td>6.5</td>
</tr>
<tr>
<td>P. arakleffeferiella sp.</td>
<td>6.5</td>
</tr>
<tr>
<td>Nilothina sp.</td>
<td>6.3</td>
</tr>
<tr>
<td>Phaenopspectra sp.</td>
<td>6.3</td>
</tr>
<tr>
<td>Procladius (Holoiana) sp. johnsoni Roback</td>
<td>6.3</td>
</tr>
<tr>
<td>Chironomus sp. pliusus gp</td>
<td>5.6</td>
</tr>
<tr>
<td>Ablabesmyia sp. verra Roback</td>
<td>5.1</td>
</tr>
<tr>
<td>Glyptotendipes (G.) sp.</td>
<td>5.0</td>
</tr>
<tr>
<td>Lauterborniella agyralllodes (Kieffer)</td>
<td>5.0</td>
</tr>
<tr>
<td>Chironomus sp. cf. commutatus</td>
<td>4.9</td>
</tr>
<tr>
<td>Polypedilum sp. betrenatum gp</td>
<td>4.9</td>
</tr>
<tr>
<td>Psectrocladius (Monopsectrocladius) calcatus</td>
<td>4.9</td>
</tr>
<tr>
<td>Edwards</td>
<td></td>
</tr>
<tr>
<td>Cladoianytsrus sp. vanderwalopi gp.</td>
<td>4.8</td>
</tr>
<tr>
<td>Djalnabatista pulchra (Johannsen)</td>
<td>4.8</td>
</tr>
<tr>
<td>Microtendipes sp. pedellus gp</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Figure 2 shows that profundal/sublittoral chironomids have different tolerances for acidification. Although many of the species are tolerant, several of them disappears when the pH decreases. That is, a prerequisite for building a monitoring system for acidification based on chironomids.

There was no correlation between pH and the number of chironomid and chaoborid species / taxa in the Kajak samples ($r^2 = 0.16$, Pearson correlation), nor between pH and the abundances in the spring and fall samples ($r^2 = 0.0001$ and $r^2 = 0.18$, respectively). This has also been shown in earlier studies for the total benthic fauna in Laurentian Shield lakes (e.g. Dermott 1985, Dermott et al. 1986).

Parts of this may be due to oxygen depletion in the hypolimnion of the lakes. The faunal composition in the spring samples indicated low oxygen content in some of the lakes, and this tendency was strengthened when the fall samples were identified. Oxygen measurements during the summer of 1998 verified the case. Most of the lakes with depletion were in the upper part of the pH gradient, although some of the lakes with lower pH also experienced low oxygen content. A correlation between the oxygen concentrations in August 1998, measured at the actual sample depths from 1997, and species richness from 1997 was significant at the 0.01 level ($r^2 = 0.49$, Pearson correlation). Also the fall abundance showed a significant correlation ($p < 0.05$, $r^2 = 0.34$), while the spring samples showed no significant correlation ($r^2 = 0.16$).

A detrended correspondence analysis (DCA) using CANOCO 4 (ter Braak and Smilauer 1998) was carried out on the quantitative data from the spring and the fall in the 18 lakes sampled in 1997. The analysis was performed with detrending by segments, abundance data were log transformed, and rare species were downweighted. The length of the gradient along the first ordination axis was 3.8 SD units indicating that a unimodal response model would fit the data best (Birks 1995).

The same abundance data was used in a canonical correspondence analysis (CCA) with pH, sample depth, dissolved organic carbon (DOC), labile aluminium (LAI) and the calcium concentration as environmental variables. The abundance data were log transformed and rare species were downweighted, and the environmental data were log transformed except for pH. The initial analysis showed as expected that pH and LAI were strongly correlated, pH had the highest inflation factor and was consequently eliminated from the further analyses. A Monte Carlo test with 999 permutations showed both the first canonical axis and all the canonical axes to be significant ($p = 0.001$ and 0.002 respectively). Foreword selection was
performed on the remaining variables, and LAI, DOC, and ANC contributed significantly to the ordination. Results of the CCA is shown in Table 3 and Figure 3.

Table 3. Results from the CCA analysis. \( r \) is the weighted correlation coefficient between the environmental variables and the first two ordination axes. Only variables that showed significant contributions \((p \leq 0.05)\) to the first axis is listed.

<table>
<thead>
<tr>
<th></th>
<th>CCA axis 1</th>
<th>CCA axis 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue (( \lambda ))</td>
<td>0.361</td>
<td>0.186</td>
</tr>
<tr>
<td>Abundance data variation explained (%)</td>
<td>19.2</td>
<td>5.2</td>
</tr>
<tr>
<td>LAI</td>
<td>( r = 0.6890 )</td>
<td>( r = -0.3961 )</td>
</tr>
<tr>
<td>DOC</td>
<td>( r = -0.6475 )</td>
<td>( r = -0.2609 )</td>
</tr>
<tr>
<td>ANC</td>
<td>( r = -0.6084 )</td>
<td>( r = -0.2011 )</td>
</tr>
</tbody>
</table>

Figure 3. CCA biplot of species and environmental variables. The variables that did not contribute significantly to the ordination is shown with hatched arrows.

The low percentage of the explained variance in the species data by the two first ordination axes (15.4 %) implies either a large amount of noise in the data, or that the environmental variables chosen were not the most important ones in structuring the chironomid society, or both. However, the eigenvalues from the DCA analysis (\( \lambda_1 = 0.488 \) and \( \lambda_2 = 0.323 \)) and the amount of variance explained by DCA axis 1 and 2 (13.8 % and 9.1 % respectively) indicates that noise is important in this ordination. One obvious factor is the oxygen depletion in some of the lakes. Another factor causing noise is the time of sampling in the spring. The chironomid emergence had started and was probably peaking when the collections were done.
Sampling exuviae in foam by the lake shores evidenced this. This means that the abundances data from the spring samples are unreliable, and a CCA analysis of the spring data alone did not give any significant ordination. Sampling of exuviae were also done during the fall fieldwork. However, then there were almost no chironomid emergence, and the actual abundance data may reflect different environmental stress in the various lakes.

Substituting the abundance data with presence - absence data, and running a CCA analysis on that data set, with a similar setting as that above, also yielded a significant ordination. The results were pretty much similar to the analysis of the quantitative data. The eigenvalues were slightly lower ($\lambda_1 = 0.315$ and $\lambda_2 = 0.170$), and the percentage of the variation in the presence-absence data explained for was 9.5 % for the first CCA axis and 5.1 % for the second axis. Performing forward selection on the environmental variables resulted in ANC, LAI and DOC as the ones giving significant contributions to the ordination. ANC was the best variable with the highest correlation with the first CCA axis ($r = 0.7671$), then LAI ($r = -0.7381$) and at last DOC ($r = 0.6199$).

**Concluding remarks**

The results from the pilot project in 1997 gives promising signals regarding chironomid communities as indicators of acidification. The chironomid project in the NLRS will continue with sampling for three years. The sampling started in October 1998, and the problem with oxygen depletion in the hypolimnion was and will be avoided by sampling in the sublittoral above the depletion. Sampling will only be done in the fall, thus eliminating the problem with early emerging chironomids in the spring. The number of lakes will be increased to 21 with the inclusion of a lake with pH at 4.5, thus increasing an already large pH gradient.

The labile aluminium concentration in the lakes will be measured in the future. This may give indications of whether it is pH itself or pH-related phenomena that actually structures the chironomid society. The present study cannot say anything about that issue.

Finally the link between acid neutralising capacity and the chironomid community may give us the possibility to estimate which species that can be expected to reappear as acidified lakes recovers. This will be based on construction of scenarios based on different emission protocols, as is done for critical loads and critical loads exceedances (Hindar and Henriksen 1998).

**Acknowledgement**

I am very much indebted to John Gunn, Ed Snaeins, Joelyne Heneberry and the rest of the people at the Coopunit, Laurentian University, Sudbury, for doing a great job with the sampling and for providing me with environmental data from the Killarney Lakes. Thanks also for the hospitality during my stay in Sudbury and Killarney in the spring of '97. Atle Hindar and Arne Henriksen, NIVA, are thanked for giving me access to their calculations of the ANC from the Killarney lakes. This study has been financed by the Norwegian Directorate for Nature Management, with Steinar Sandøy as coordinator.

**References**


12. The Canadian Wildlife Service Acid Rain Biomonitoring Program - Monitoring and Modelling the Effects of Acid Rain on Water Birds in eastern Canada

Don McNeill, Canadian Wildlife Service (Ontario Region), Canada

The Acid Rain Problem in Canada - History and Current Status

The problem that acid rain posed to Canadian lakes and forests was identified in the 1960s, but it was not until the late 1970s that governments funded research to determine the extent and magnitude of the problem in eastern Canada. Results of studies in the 1980s, focused largely on gamefish and gamefish lakes, led to the concept of the target load of 20 kg wet sulphate (SO₄) deposition/ha/yr to protect aquatic resources. By 1987, the Canadian Wildlife Service (CWS) started its Acid Rain Biomonitoring Program, following 7 years of cause-effect research that linked acid deposition to changes in aquatic food webs, habitat use and breeding performance of aquatic birds. In 1990, both Canada and the United States (U.S.) prepared "science assessments (essentially summaries of the state-of-knowledge on acid rain at the time)" as supporting science documents for the Canada/U.S. Air Quality Agreement that was signed in 1991. By the end of 1995, Canadian emissions were 43% lower than in 1980, surpassing sulphur emission targets.

In 1997, a second science assessment was conducted in Canada; the Aquatic Effects part of that document (Jeffries, D. S. 1997) relied heavily on CWS chemical and biological data, as well as modelling efforts. A reassessment of the chemical data from eastern Canada showed that the regional status of lakes and their sensitivities had changed little since 1990. There was evidence of a decline in both deposition and lake water concentrations of SO₄, but there was also an unexpected, substantial and off-setting decline in base cations (notably Ca and Mg) in precipitation and in lakes. Of 202 lakes monitored across eastern Canada since the early 1990s, 33% were less acid, 11% were more acid, and 56% had not changed significantly in acidity status. What this suggested was that chemical recovery was much slower than expected or hoped for. The most striking improvements were seen in the Sudbury area, where damage to lakes had been particularly severe due to sulphur dioxide emissions from local smelters. Moreover, the 1997 Assessment noted that the effects of nitrogen deposition remained largely unknown, but that they had the potential to undermine some of the gains made by reducing SO₄ deposition.

Also from this assessment, several key points were stressed based on biological studies of the effects of acid rain. First, it was reaffirmed that biodiversity in aquatic ecosystems was highest near pH 6.0, and below this value, there was some loss of aquatic organisms (see Figure 1). As well, food chains were altered by acidification, becoming simpler and dominated by acid-tolerant organisms, and this process was shown to affect aquatic birds up the food chain, both through loss of preferred prey species and reduced nutritional value (reduced availability of Ca in prey from acidic lakes). These effects, and the finding that chemical recovery is much slower than hoped, collectively mean that biological recovery is slow and unpredictable (due to hysteresis), and that increased attention must be given to interactions with other potential environmental stressors, such as toxins (e.g., mercury), UV-b and climate change.

The 1997 Assessment confirmed that critical loads for a lake ecosystem is the maximum yearly amount of wet SO₂ deposition that will allow 95% of the lakes within the ecosystem to maintain a pH of 6 or more. Given the current emission targets after full implementation (year 2010), the total area of eastern Canada where deposition is expected to exceed the critical loads covers almost 800,000 km², includes about 95,000 lakes, and extends from central Ontario through southern Quebec and across much of the Atlantic provinces. Within these areas, populations of many species (fish, zooplankton, etc.) will disappear entirely from some lakes and be severely reduced in others. Altogether, it is estimated that continuing
acidification of these lakes will result in a net loss of nearly 162,000 fish populations. Extending protection to all of these vulnerable ecosystems will require further deep cuts in sulphur dioxide emissions in Canada and the U.S.; an estimated additional 75% cut in emissions (beyond current 2010 targets) would be necessary to bring wet $SO_2$ deposition levels below the critical loads for virtually all aquatic ecosystems in eastern Canada.

**Surface Waters at Risk in eastern Canada**

Of nearly 900,000 water bodies in eastern Canada south of 52° N latitude, 72% lie in areas of low buffering; 56% of these are small (<5 ha) mostly in sensitive areas in the provinces of Quebec and Ontario (see Figure 2). These small lakes and wetlands are especially vulnerable to the effects of acidification, and are of particular concern because they represent the most important habitats for wildlife, including waterbirds. For example, nearly 192,000 pairs of ducks and common loons (**Gavia immer**) nest in acid-sensitive areas of Ontario which receive considerable acid loadings (>10 kg/ha/yr wet $SO_2$ deposition; see Figure 7).

Using the chemical database (1980-1995 data on 4911 lakes) compiled for the 1997 Assessment, GIS maps showed the general distribution of pH across much of eastern Canada (modelled area ca. 1.4 million km$^2$). Approximately 31% of this land mass (440,000 km$^2$) supports lakes with pH above 7, 43% (660,000 km$^2$) supports lakes with pH 6 to 7, 25% (350,000 km$^2$) has lakes with pH 5 to 6, and 1% (14,000 km$^2$) supports lakes with pH below 5.0. Regionally, Ontario is dominated by high pH areas (>6.5) in the north and west, with parts of central Ontario, much of southern Quebec and Atlantic Canada characterized by lower pH (<6). Pockets of low pH along the north shore of the St. Lawrence River in Quebec and in Atlantic Canada are in part due to natural organic acidity.

**Acid Rain and Ecological Effects in eastern Canada**

From an acid rain perspective, healthy lakes are those with pH values above 6 and generally moderate to high acid-neutralizing capacity (ANC - buffering), so they are able to withstand acidic inputs with few changes in aquatic fauna (Figure 1). Damaged lakes are those with limited to moderate buffering, generally between pH 5 and 6 and which have usually lost some biota that inhabited the lake(s) prior to acidification. Further acidic inputs will usually increase the damage in these lakes. Acidic lakes are those with pH below 5 and with no ANC. These lakes have substantial to severe damage to their aquatic food chains, almost always having lost populations of both fish and acid-sensitive invertebrates, and generally retain a simple, acid-tolerant food web dominated by large, predatory macroinvertebrates.

Across eastern Canada, species loss curves derived from various data sources support this general pattern, in that for benthic invertebrates, zooplankton and fish, significant loss of species usually begins around pH 6 and becomes very pronounced below pH 5 (see Doka et al. 1997).

**Canadian Wildlife Service Acid Rain Blomonitoring Program**

To verify the degree of environmental improvement achieved and the adequacy of acid rain control programs in North America, CWS undertakes integrated wildlife, food chain and aquatic chemistry monitoring. This program is designed to collect sufficient long-term ecological data to evaluate (at several spatial and temporal scales) the recovery of acid-sensitive lakes and wetlands that are expected to respond to reduced acid deposition (see McNicol et al., 1995).

**Waterbird Guilds** - Three types of waterbirds are studied - piscivores, dabbling ducks and diving ducks. Piscivores are fish-eating birds that are typically found on large lakes and rivers. CWS studied primarily the common loon (**G. immer**) and the common merganser (**Mergus merganser**). Lake acidification affects
the piscivore guild more directly than any other bird group, because fish populations, the main prey of piscivores, are either reduced or lost in acid-stressed lakes.

Figure 2. CWS study sites in eastern Canada.

The dabbling ducks are omnivorous birds that can be found on all types of habitats, but are typically associated with wetlands. This group includes the mallard (*Anas platyrhynchos*), the American Black Duck (*Anas rubripes*), the Wood Duck (*Aix sponsa*) and the Green-winged Teal (*Anas crecca*). Across most of the acid-stressed regions of eastern Canada, the black duck is the most common dabbling duck. During the breeding season, female and young dabbling ducks forage heavily on aquatic invertebrates. This guild is very adaptable and can use a wide variety of food resources available in all types of habitats, but lake acidification reduces the quality and diversity of their invertebrate prey.

The diving duck guild is composed of typically insectivorous ducks that use all types of habitats, but are often associated with small lakes. This group includes the common goldeneye (*Bucephala clangula*), the hooded merganser (*Lophodytes cucullatus*) and the ring-necked duck (*Aythya collaris*). These ducks also forage primarily on aquatic invertebrates through the breeding season, and their distribution and breeding success is influenced by the availability and quality of invertebrate prey in acid-stressed lakes.

**Waterbird Habitats** - Three main types of habitats are studied - wetlands, small lakes and large lakes. Wetlands may be any size, but are usually small (< 5 ha) and take the form of marshes, swamps, bogs, fens, beaver ponds or forested swamps. These are key waterbird and wildlife habitats, and often act as sinks for sulphur, controlling chemistry of downstream water bodies. Small glacial lakes are common on the Precambrian Shield and are preferred by several types of waterbirds. Many of these lakes are very sensitive to acid inputs because they have small watersheds that are situated on granitic bedrock which offers little buffering. Hence, these lakes may acidify quickly and require many years to recover. The large, oligotrophic lakes are important for large sportfish and were the first lakes in which the acid rain problem was recognized. These lakes are important for certain waterbirds, notably the piscivores, and have typically been the most studied by government agencies.

**Study Areas and Study Site Characteristics** - The CWS conducts acid rain studies in 5 main areas of eastern Canada (Figure 2). Two sites, the Lepreau and Kejimkujik study areas (total 101 lakes), both situated in the Atlantic Maritime Ecoregion. Three sites are located in Ontario (ON); Algoma, Muskoka and Sudbury, all of which are located in the Boreal Shield Ecoregion. Collectively, more than 600 water
bodies are monitored in Ontario by the CWS. In addition, about 1600 lakes are monitored by volunteers through the Canadian Lakes Loon Survey.

In Ontario, CWS lakes tend to be small (< 20 ha, often headwaters), cover a broad pH range, and include extremely damaged lakes near Sudbury. About 40% of the Ontario CWS lakes are fishless. Atlantic CWS lakes in the Lepreau area are similar to lakes in Ontario, while the Kejimkujik Park lakes tend to be large (> 40 ha), with fish, and acid-stressed. Overall, the Atlantic lakes are very sensitive (very low calcium levels) and generally have a strong influence of natural, organic acidity (high DOC). In Ontario, the Canadian Lakes Loon Survey lakes are typically large (> 40 ha) and clear, with most above pH 6.

Recent Trends in Ontario CWS Biomonitory Lakes

Chemical - For the three Ontario study areas pooled, trend analyses on chemical data collected between 1988-1997 suggests that about 70% of the lakes have shown no significant change in acidity status (pH or alkalinity), while 25-30% of the lakes have shown some significant improvements, and a few lakes have gotten worse (Figure 3). Half of the lakes have exhibited significant declines in base cations, and 40% have exhibited significant declines in SO$_4$; it is this decline in base cations that is thought to have offset the reduction in SO$_4$, thereby minimizing the anticipated improvements in pH or ANC. Note that there has been little change in nutrient-related parameters such as total phosphorus or dissolved organic carbon.

Waterbirds - In Ontario, 3815 lake-years of records suggest that habitat use differs among the major waterbird guilds (Figure 4). Piscivore indicated breeding pairs (dark bar) and broods (light bar) show proportionally higher use of high pH lakes, whereas the dabbling guild shows a relatively uniform distribution of habitat use across the pH range, perhaps with slightly higher use of mid-pH lakes. The diving duck guild shows a distribution skewed opposite to piscivores, that is, higher use of low pH lakes (< 5.5) by IP with broods showing a more uniform distribution. The relatively high occurrence of common golden-eyes on low pH lakes near Sudbury has a strong influence on the pattern exhibited here.

Development of Waterfowl Logistic Models

Our modelling work has focused on three indices of waterbird reproduction: the occurrence of breeding pairs (measures suitability of nestng habitat), the occurrence of broods (measures suitability of brood-rearing habitat), and the survival of common loon two-chick broods (measures specific suitability of
breed-rearing habitat for loons, a key indicator species for acid rain). Each of these dependent variables is influenced by the physical and chemical characteristics of lakes slightly differently.

Because dependent variables had binary values, we used logistic regression to develop models for predicting these reproductive parameters. We used backward, stepwise selection procedures, weighted for the number of surveys on each lake. The models developed for piscivores (loons and common mergansers) had the best fit and most variation explained. This can be attributed to the fact that pH and lake size have a strong effect on the occurrence of fish, which in turn directly influences the probability of having fish-eating birds nest on the lake. Because of this clear and direct relationship, we used piscivore models to demonstrate effects of emission reductions on aquatic biota, although models for other waterbird guilds (species) have also been developed. In general terms, the ability of habitat to support breeding piscivores depends both on area and pH (Figure 5). Larger and higher pH lakes are more likely to support breeding pairs (IP) and broods than smaller or more acidic lakes. For example, a 10 ha lake with pH 7 has a 50% chance of supporting a breeding pair of piscivores, a 30% chance of supporting a loon brood, and 80% chance of having 2 loon chicks survive to fledging stage.

*Figure 5. pH and piscivore models.*

*Can we predict the eventual benefits of SO\textsubscript{2} emission controls on the suitability of waterbird breeding habitat in eastern Canada?*

**Waterfowl Acidification Response Modelling System (WARMS)**

To model the effects of SO\textsubscript{2} emission reduction scenarios on waterbirds, we used the Waterfowl Acidification Response Modelling System (WARMS). WARMS is a computer software package that consists of a series of linked models: it links sulphate (SO\textsubscript{2}) deposition (secondary watershed level) to lake chemistry (pH or alkalinity), and fish and wildlife models (Figure 6). Input to WARMS consists of lake files (chemical and physical characteristics), characteristics of secondary watersheds (runoff coefficients, and wet, dry and background S deposition), predicted changes in SO\textsubscript{2} deposition (from atmospheric models) based on emission scenarios, and logistic or linear fish and waterbird models to estimate effects of changes in acidic deposition on habitat suitability for selected biota, as well as providing eventual pH and alkalinity for those lakes. The acidification model is the Cation Denudation Rate Model (Steady State), modified for DOC (Marmorek et al. 1996).

*Figure 6. WARMS model process.*
**Wet $SO_\text{4}$ Deposition**

Revised wet $SO_\text{4}$ deposition values (kg/ha/yr) were generated from atmospheric receptor site data provided by the Atmospheric Environment Service of Environment Canada (S1: 1982-1986; S2: 1990-1993; see Figure 7). Interpolated maps for northeastern North America show that levels of $SO_\text{4}$ deposition have generally decreased and will continue to do so under the current legislated sulphur emission cutbacks, specifically after Canadian controls (S3: ca. 40% cut by 1994, achieved) and after U.S. controls (S4: ca. 40% cut by 2010). Southern Ontario typically receives the highest deposition of $SO_\text{4}$, but a broad region of central Ontario and Quebec receives high levels as well. Under each of the emission reduction scenarios, improvements occur in most regions, except that Atlantic Canada receives relatively little benefit until the 2010 emission cutbacks, indicating that much of the Atlantic deposition problem comes from U.S. emissions. Much of southern Ontario and Quebec will continue to receive 15-20 kg/ha/yr $SO_\text{4}$, which is below the target load set in the mid-1980s, but which exceeds the critical loads for many sensitive waters in this region.

### Figure 7. Measured and modelled wet $SO_\text{4}$ deposition in eastern North America.

**Predicted pH and Habitat Suitability Change in eastern Canada**

We used the current chemical database (4911 lakes), the logistic models developed using CWS data, and the $SO_\text{4}$ deposition scenarios (S1-S4) described above as input to WARMS, and then we ran models to estimate pH change and piscivore habitat suitability change across eastern Canada under these scenarios.

**pH** - For pH, WARMS modelling results showed that most chemical changes (expressed as % of affected land mass) would occur in areas where current lake pHs are between 5 to 6 (Figure 8); no change would be expected for lakes above pH 7, and minimal improvements are expected for lakes with pH < 5. In fact, further emission reductions of 75% from 2010 targets should result in over 80% of eastern Canada supporting lakes with pH above 6 (i.e. above critical loads levels).

### Figure 8. Modelled pH change in eastern Canadian lakes.
In examining regional chemical change, we arbitrarily defined "levels" of pH change as follows: no change - a change of 0, or a slight decline in pH (<0.1 units); minor increase - a pH improvement of 0.01 - 0.10 units; small increase - 0.1 - 0.2 pH units; large increase - > 0.2 pH units. Under the 1994 emission scenario (S3), minor increases would occur in 44% of eastern Canada (throughout central Ontario and most of Quebec), with small pockets exhibiting small increases (2%), but there would be no large increases in pH, and no change through most of the area examined (54%), particularly the Atlantic region. With the 2010 (S4) scenario, large increases (3%) would be observed in affected regions in central Ontario and western Quebec, with small increases (12%) occurring throughout much of the remaining acid-sensitive regions of Ontario and Quebec. As well, Atlantic Canada, particularly Nova Scotia, would experience minor increases in pH (39% of total land mass). With the recommended further 75% reduction in emissions from 2010 levels, most of central Ontario and vast regions of Quebec would experience large pH improvements (29%), as would portions of southern New Brunswick. Collectively, lakes in 66% of the modelled area would be expected to show some improvement in pH.

Piscivores - Applying the piscivore breeding pair model to the acidification model projections above provides estimates of changes in the suitability of lakes to support nesting piscivores (common loons and common mergansers) across eastern Canada (Figure 9).

![Figure 9. Maps of improvement in suitability of nesting habitat for piscivores (common loons and common mergansers) between 1982 and 2010 scenarios (left), and 1982 and 75% further emission reduction scenarios (right). Dark represents no change, medium is small improvement, and light is large improvement.](image)

Categories of change were set as: no change - <0.5% increase in suitability between scenarios; small improvement = 0.5-1.5% increase between scenarios; large improvement - >1.5% increase between scenarios (maximum recorded was 9.2%). Comparing predicted changes in habitat suitability between 1982 levels (steady state) and full implementation of U.S. controls (2010), no change was projected through most of eastern Canada (73%), particularly Atlantic Canada, with small improvements predicted to occur in 23% of the region, notably through much of Quebec and central Ontario. Isolated pockets of large improvements (4%) are projected to occur, especially near Sudbury and Rouyn-Noranda (two areas of historically high, point source emissions). With a further 75% emission reduction, however, large improvements would dominate changes (29%), most noticeably through central Ontario and most of Quebec, with small improvements occurring through much of Atlantic Canada (20%).

Collectively, the projected changes in pH and changes in habitat suitability (expressed as the % of affected land mass) confirm that each emission scenario results in some improvements to the chemical conditions and nesting habitat suitability of water bodies in eastern Canada (Figure 10). A large proportion of the land mass will not change, either because lakes in certain areas are well-buffered, or do not receive enough deposition to cause chemical deterioration. As well, for nesting piscivores, many
lakes will not change in suitability simply because they have characteristics that make them unsuitable for nesting (e.g. too small, too shallow), irrespective of lake chemistry.

What is clear, however, is that even with the 2010 scenario, relatively few, large scale improvements can be expected across much of eastern Canada. Instead, it will require further reductions to bring about broad scale, significant chemical and biological improvements.

![Graph showing pH improvements and habitat suitability improvements.](image)

*Figure 11. Patterns in waterfowl numbers.*

*Figure 10. Anticipated change in pH and habitat suitability.*

**Observed vs. predicted waterbird responses**

Our models predict that piscivores should prefer higher pH lakes, and diving ducks, notably the common goldeneye, should prefer low pH lakes. In both cases, the birds probably respond to the presence of fish: piscivores like high pH lakes because they support healthy fish populations, and goldeneyes like low pH lakes because they often lack fish but support abundant, acid-tolerant invertebrate prey. Thus, with increasing lake pH, we expect fish to return to lakes (Figure 1), and correspondingly we should see increases in the breeding density of piscivores and decreases in the insectivorous goldeneye. In Ontario, CWS has been conducting population surveys of waterbirds in the historically acid-damaged Sudbury region since the mid 1980s. Chemical, and perhaps biological, improvements are expected to occur more rapidly at Sudbury because of major point source emission reductions over the past 20 years. In support of the model predictions, waterbird monitoring near Sudbury has demonstrated significant increases in numbers of breeding pairs of common loons, hooded mergansers and all piscivores combined (Figure 11), and significant decreases in numbers of common goldeneyes. Thus, patterns in numbers of local breeding waterbirds are in the direction expected if chemical and biological improvements are occurring in the Sudbury area.
Conclusions

Acid rain poses a serious threat to wildlife (notably waterbirds) in eastern Canada through a variety of ecological and ecotoxicological processes that occur at lower trophic levels, but which ultimately cause reproductive impairment and/or shifts in habitat selection or diet. The CWS Acid Rain Biomonitoring Program monitors ecological responses of waterfowl, loons and their foods to a changing acid deposition environment to verify spatial and temporal aspects of the biological recovery (status) of acidified, damaged or susceptible aquatic systems. Modelling is an extremely important component of the program because there is no direct means of predicting the nature and extent of aquatic ecosystem recovery from acidification. Results from monitoring and modelling efforts together demonstrate that certain waterbirds (especially piscivores) are effective indicators of acidification, and that little progress has been realized to date in chemical and biological recovery of lakes. Our predictions demonstrate that little improvement in habitat suitability for waterbirds will occur in eastern Canada even with the strongest legislated emission reductions (post 2010), but that further recommended reductions should lead to substantial improvements in some populations, as currently being observed in the heavily damaged (but recovering) Sudbury area. Further development, validation, and expansion of regional waterbird models (and other biological models), together with ongoing population and production monitoring, is necessary to improve confidence and accuracy in habitat suitability model predictions and observed progress.

References


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Appendix A. Programme

Tuesday 13. October, 1998

0900-0930 Registration

0930 Opening

Chairman: Bjørn Olav Rosseland

Large scale monitoring: Aims, possibilities and realistic approaches, harmonising, sampling strategy, data processing and evaluations
Gunnar G. Raddum, University of Bergen, Norway

The acidification indexes. Simple and robust tools for monitoring?
Gunnar G. Raddum, University of Bergen, Norway

Strategies for fish monitoring at different environmental stress scenarios
Bjørn O. Rosseland, NIVA, Norway

1105-1130 Coffee

Comparison of different indices used in the 95 national survey of lakes and streams in Sweden
Lars Eriksson, Dept. of Environmental Assessment, Sweden

Acidification in the Vosges Mountain (North-eastern France). Assessment of biological status and trends by use of different methods
Francois Guerold and Olivier Dangles, University de Metz, France

Invertebrate communities of high mountain lakes (Tatra Mts) as acid pollution indicator

1230-1430 Lunch

Chairman: Jim Bowman

Networking of long-term integrated monitoring in terrestrial system, NoLIMITS
Ian C. Simpson, Environmental Change Network, UK

Comparison of chemical and biological data
Jim Bowman, Environmental Protection Agency, Ireland

Acidification induced changes in nutrient status of Tatra Mountain lakes
Evzen Stuchlik, Charles University, Czech Republic

Biodiversity as a monitoring method of polluted river
Andrzej Kownacki, K. Starmach Inst. of Freshw. Biol., Poland

Chironomids in lakes with different pH in Killarney Provincial National Park, Canada, The Northern Lake Recovery Study (NLR3)
Godtfred A. Halvorsen, University of Bergen, Norway

Stream Macrozoobenthos Monitoring in Lithuania: Methods, Results and Problems
Kestutis Arbaciauskas* and Liutauras Stoksus**
* Institute of Ecology, Lithuania
** Vilnius University, Lithuania

1530-1600 Coffee
Hydrobiological monitoring in Latvia under the ICP Waters and ICP IM programmes
The Canadian Wildlife Service Acid Rain Biomonitoring Program - Modelling and Monitoring
the Effects of Acid Rain on Water birds in eastern Canada
Donald K. McNicol, Environment Canada, Canada

Posters:

Biological methods in the monitoring of streams in the Berezinsky Biosphere Reserve
Gennadij Tishchikov, Republ. Radiation Control and Environm. Monit. Centre, Belarus
Appendix B. Participants

Participants at the joint ICP Waters and ICP IM
"Workshop on biological assessment and monitoring; evaluation and models"
October 13th 1998, Zakopane, Poland

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Appendix C. Reports and publications from the ICP Waters Programme


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