PART TWO: SURVEYS & ASSESSMENTS

4.A HOW URBANIZATION AND INDUSTRIES INFLUENCE WATER QUALITY

Quantification of pollution is necessary for evaluation of environment quality and elaboration of a successful strategy for water quality improvement. Reduction of threats resulting from pollution is always the first step to implement ecological measures in IWM.

The objective of this chapter is to:

- introduce possible treats resulting from the impacts of urbanization, industrialization and agriculture;
- give a review of the assessment procedures for point and non-point pollution and identification of „hotspots" in a catchment.

Fig. 4.1
Urban sprawl often creates poverty belts that heavily impinge on water quality (photo: V. Santiago-Fandino)

IMPACT OF URBANIZATION, INDUSTRIALIZATION AND AGRICULTURE ON WATER RESOURCES

In the next decades, water shortages resulting from both a lowering of water quantity and quality will be the most urgent problem for 80% of the world's population. Development of agriculture, industry and urbanization will result in increasing water use, generate more pollutants from both municipal and industrial uses of water, and contribute to the decline of water resources quality. This may result in the limitation of water resource use by people living downstream, as well as very often degradation of the whole basin-river-reservoir system occurs. Box 4.1 presents an overview of the key sources of pollution impacting fresh waters.

BOX 4.1
The simplified impact of human activity on water resources

URBANIZATION-MUNICIPAL

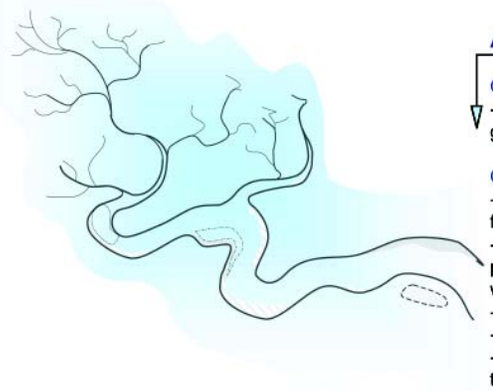
Quantity
- decrease of natural flow from fresh water and ground water uptake for drinking water purposes;
- decrease of aquifer recharge through related catchment urbanization;

Quality
- discharge of sewage effluents;
- dicharge of toxic substances during storm flash floods;
- changes in temperature regime;

INDUSTRY

Quantity
- decrease of natural flow by fresh water and ground water uptake for technological processes;
-water level decreases, e.g., due to drainage for mining, electricity generation;

Quality
- discharge of toxic substances;
- discharge of organic and inorganic pollutants;
- discharge of hot water;



AGRICULTURE

Quantity
- decrease of natural flow from fresh water and ground water uptake for irrigation and farming;

Quality
- point source pollution - discharge of manure from farms to water bodies;
- diffuse pollution – leaching of fertilizers, pesticides and manure to ground waters and fresh waters;
- outflow from aquaculture farms;
- increase of erosion;
- secondary effects: salinity of soil throughevapotranspiration;

TABLE 4.1
Potential influence of sewage outflow on freshwater quality.

POINT SOURCE POLLUTION										
	hydrological regime	temperature regime	nutrients (p;n)	BOD	suspended solids	heavy metals	toxic hydrocar-bons	salinity	acidity	pathogens
Municipal sewage	+ + +	+ +	+ + +	+ + +	+ + +	+ +	+	+ +	+ +	+ + +
Storm water	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+
Industry										
Mining	+ + +	+	+	+	+ + +	+ +	+	+ + +	+ + +	-
Power plant	+ + +	+ + +	-	-	-	+	-	-	-	-
Refinery	+	+	+	+ + +	+	-	+ + +	+	+	-
Metallurgy	++	+ +	-	-	+	+ +	+	+ +	+ +	-
Pharmacy	+ + +	+ +	-	+ +	+	+ + +	+ +	+	+	-
Chemical	+ + +	+ +	+	+ +	+	+ + +	+ + +	+	+ + +	-
Textile	+ + +	+ +	+	+ +	+ +	+ + +	+ + +	+ +	+	-
Food industry	+ + +	+ +	+ + +	+ + +	+ + +	-	+	+	+	+ + +
Agriculture										
Animal farm	+	+	+ + +	+ + +	+ + +	-	-	+	+	+ + +
Large fish farm	+	+	+ + +	+ + +	+ + +	-	-	+	+	+ + +

NON-POINT SOURCE POLLUTION (DIFFUSE POLLUTION)										
	hydrological regime	temperature regime	nutrients (p;n)	BOD	suspended solids	heavy metals	toxic hydrocar bons	salinity	acidity	pathogens
Agriculture										
Natural fertilizers	-	-	+ + +	+++	++	-	+	+	+	+ + +
Artificial fertilizers	-	-	+ + +	+	-	+	+	+ +	+ +	-
Pesticides	-	-	-	-	-	+ + +	+ + +	+	+	-

Risk /influence	+++ HIGH	++ MEDIUM	+ LOW	- NONE

IDENTIFICATION OF POTENTIAL IMPACTS ON FRESHWATER QUALITY

The first step in the assessment of human impacts on freshwater resources requires identification and quantification of the impacts of major past, ongoing and planned projects (activities) in a catchment. This allows for the recognition of potential pollutants in the basin.

Table 4.1 helps to check the potential risks in a catchment. The following three steps are proposed to

optimize the evaluation procedure for pollution risk assessment at a catchment scale:

- screening – identification of hot spots (map-study & questionnaires);
- direct assessment of impacts (field study); and
- quantification of problems (laboratory assessment and timing of monitoring).

SCREENING

The screening for identification of „hot spots" can be done based on map information, statistical analysis and the use of a questionnaire.

What are the main objectives of screening?

- analysis of the distribution of activities that affect water quantity and quality at the catchment scale;
- identification of potential pressures;
- identification of „hot spots" and elaboration of guidelines for further assessment; and
- integration of the information at the catchment level.

What issues should be identified?

- **water demand** by agriculture, municipal and industrial users:
  - **daily uptake** (m^3 day^{-1}) can be estimated using information from the water supply system or surface/groundwater usage;
  - **if there is no water supply system** - water demand should be calculated by multiplying the number of people and animals by average consumption values;
- **Potential impacts on water quality:**
  - information about sewage **outflow and sewage treatment** in a catchment should be collected;
  - **if there is no quantitative data**, it can be assumed that sewage outflow equals water use in a catchment,
  - collect information about local **industries**, including planned and implemented projects and estimate their impact (Table 4.1);
  - collect information about large **farms** and aquaculture farms;

TABLE 4.2
The global change in water consumption (from 1950 to 2000)

	1950		1980		1990		2000	
	km^3 y^{-1}	%	km^3 y^{-1}	%	km^3 y^{-1}	%	km^3 y^{-1}	%
AGRICULTURE	1130	82,7	2290	69,0	2680	64,9	3250	62,6
INDUSTRY	178	13,0	710	21,4	973	23,6	1280	24,7
MUNICIPAL	52	3,8	200	6,0	300	7,3	441	8,5
RETENTION/HYDROPOWER	6	0,5	120	3,6	170	4,1	220	4,2
Total	**1366**	**100**	**3320**	**100**	**4130**	**100**	**5190**	**100**

(after Shiklomanow, 1993)

TABLE 4.3
The consumption of water in litres per capita per day

Developing countries without access to piped water	Countries with access to a water supply system or effective private well	Countries with concentrated water supply system and introduction of water taxes (water price)
50 - 150	200-300	120-160

- calculate the number of cows, sheep and pigs per 100 ha of agriculture land and
- identify regions with high **impermeable** areas, especially in urbanized regions.

- Make aggregations of your information and mark „hotspots" on maps of 1:50 000 or 1:100 000 resolution for catchments smaller then 100 km^2, and 1:500 000 for catchments greater than 100 km^2.

DIRECT ASSESSMENT OF IMPACT

After identification of „hotspots" the assessment should be continued in the field according to the following steps:

- establishing **monitoring stations**:
  - **for point source pollution** set at least three stations, located at:
    - outflow of sewage;
    - 100-1000 m upstream of the inflow; and
    - 100-1000 m downstream depending on river width.
  - **for diffuse pollution:**
    - at least two stations should be chosen based on a land-use map and for river sections of between 10 to 50 km.
- as a first step, conduct simple field **measurements** at the identified stations at times of low and high discharge and taking into account the timing of sewage discharge:
  - **physical measurements** such as: temperature, O_2 concentration, pH, conductivity, salinity;
  - **preliminary** chemical measurements should be considered;
  - use of **bioassessment** methods that will give an indication of the possible toxic effect of the sewage inflow (for more information - see chapter 7.D);
  - use **biomonitoring** methods (e.g., macroinvertebrates, macrophytes, fish) to check the effect of sewage inflow on a river eco system (for more information - see chapter 6.A);
- **compare the data** for upstream and downstream stations. If differences are greater than 20% in the measured values, contacting a professional laboratory to focus on the problem should be considered.

The timing of monitoring is important, especially if you have information about illegal sewage inflows and stormwater pollutants (dilution effects alter very high concentrations of pollutants).

QUANTIFICATION OF THE PROBLEM

The preliminary issues identified during the direct assessment of impacts should be further specified by chemical analysis made in a professional laboratory. Water samples for laboratory study (1-5 litres) should be collected at each study station.

- the water should be taken from the **main course** of a river. If the river is more then 10 m wide, an integrated sample should be collected by mixing equal volumes of water from every 10-20 m of the cross section;
- **transport** the samples to the laboratory or use field equipment to make analyses
- standardize the system; and
- the basic **parameters** that should be determined include: BOD_5, Total Suspended Solids (TSS), Organic Suspended Mater (OSM), Coliform index, NH_4-N, NO_2-N, NO_3-N, N tot, PO_4-P, P tot, TOC;

To quantify the impact of pollution on water quality, compare the obtained data with data from non-impacted (reference) sites. The data can be compared to regional standards for water quality.

To have a complete picture for formulating a management strategy and identification of „hot spots" in the catchment, aggregation of all the above information can be made by using one of the following systems:

- GIS (see chapter 4.B);
- hydrochemical profiles of a river; and
- use of water quality indexes.

4.B. HOW TO ASSESS LANDSCAPE IMPACTS ON WATER QUALITY

A landscape is the framework for all biogeochemical and ecological processes. Its structure defines the rate of water and chemical exchanges between land and water ecosystems in catchments and species biodiversity. All these factors determine the self-regulating potential of ecosystems and their resilience to human impacts. This chapter presents basic methods of landscape assessment using aerial photography and remotely sensed imagery in conjunction with GIS technology for estimating the rate, direction, and possible impact of landscape changes on natural resources, such as water and biodiversity.

Fig. 4.2
The Lubrzanka River Catchment in the Swietokrzyskie Mountains of Poland (photo: Department of Applied Ecology)

HOW ARE LANDSCAPES ASSESSED?

From the point of view of protection and management of natural resources it is important to identify and quantify the following landscape structures (Table 4.4):

TABLE 4.4
Role of various structures in a landscape

STRUCTURE TYPE	FUNCTION IN A LANDSCAPE
built-up areas	create impermeable surfaces in the catchment and act as a pollution source, and a place for humans to expand
agricultural lands	are sources of non-point pollutants and the cause of biodiversity loss
linear anthropogenic structures	source of pollution and also participate in landscape fragmentation
meadows	are buffers of medium effectiveness against pollutants
woods and forests	efficient structures regulating rate and mode of flow of water and chemicals through a landscape
land / water transition zones	regulate the exchange ratio of water and nutrients between water and terrestrial systems
water bodies	act as both a recipient of pollutants and as a tool for reduction of nutrient content in ground water

One of the most efficient methods for identification of these areas, and therefore estimation of their role in water and nutrient circulation, is **teledetection**, a method based on recording, visualization and analysis of electromagnetic radiation. Teledetection incorporates classic aerial photography, based on visible light, and recording and visualization of other parts of the electromagnetic spectrum. The type of electromagnetic radiation used for analysis of landscape structures influences the type of information gained and, therefore, one's ability to interpret data - **photointerpretation** (Table 4.5).

HOW ARE LANDSCAPE STRUCTURES IDENTIFIED ON AERIAL PHOTOGRAPHS?

Aerial photography provides large amounts of information about the area of interest including: physical profile, freshwater distribution and its quantity and quality, distribution and biomass of vegetation and characteristics of human impacts. There are, however, two points, that must be emphasized:

- to find and properly interpret the information, **direct observations** in the landscape have to be carried out; and
- **knowledge** about an area has to be obtained and a clear goal of the survey has to be set prior to the analysis of photographs.

TABLE 4.5
Types of electromagnetic radiation used for landscape assessment.

RADIATION TYPE	WAVE LENGTH [m]	OPTIMAL USE
gamma	$<10^{-11}$	for geological purposes - detection of ores, e.g., thorium, potassium, uranium
ultraviolet	$10^{-8} – 4*10^{-7}$	mostly for receiving pictures comparable with classical photography, also for detecting objects characterizing spontaneous or triggered luminescence
visible light	$4*10^{-7} – 7*10^{-7}$	i) grey scale photography: - orthochromatic – sensitive to all spectrum of visible light except red light - orthopanchromatic – sensitive to all colours - superpanchromatic – with increased sensitivity to red light ii) colour photography - classical - multispectral – allow precise colour differentiation (for analysis of vegetation types, water pollution, etc.) the most popular type of aerial photography (low cost, easy interpretation) analysis of land geology and morphology, changes in shapes of river channels, estimation of extent of different areas, plant cover and biomass analysis, calculation of river discharges, crops, erosion rate, etc.
infrared	$7*10^{-7} – 3*10^{-4}$	precise vegetation cover and soil type analyses, identification of water in the landscape [diminishes shade effect]
Medium and long wave infrared	$3*10^{-4} – 10^{-2}$	mostly night pictures – analysis of land structure, especially in arctic regions and volcanic areas, detection of structural anomalies in land geology, estimation of forest conditions, fire risk, in hydrology: analysis of soil moisture, ice cover development, influence of urbanization and industrialization on water pollution, and finally localization of oceanic currents
microwaves	$10^{-2} – 3*10^{-3}$	analysis of meteorological phenomena
radar detected radiation	$7{,}5*10^{-3} – 1*10^{-1}$	cartography, geological studies, topography analyses [slopes], hydrology: analyses of soil moisture, drainage structure, snow and ice cover

Topographic profile

The best source of information about the physical profile of the land is a stereoscopic picture. Analysis of such pictures is possible only with a stereoscope. With some experience you may also try to identify some landscape structures on common photographs. Some of the forms will be easily analyzed and quantified on large-scale pictures [1:50 000], while some require use of smaller scales, like 1:10 000. In some cases, guides for identifing landscape forms maybe useful.

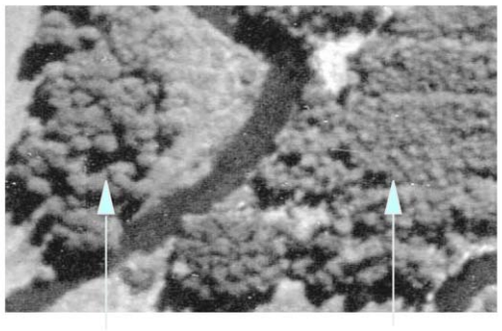
Forests, woodlands, bushes

Identification of woodlands is possible as they differ from the background by their colour and grainy structure. Species identification may be conducted on the basis of the separation of single trees and their shadows.

In temperate climates, forests are composed of coniferous and deciduous species. On a photograph the difference between the groups is sometimes visible as **differences in colour brightness** - coniferous forests are darker and the **size of the grains,** reflecting the heads of trees, for conifers are smaller (Box 4.2).

BOX 4.2

How are deciduous and coniferous forests identified?



bigger grains reflects bigger heads of deciduous species

smaller grains are usually indicators of conifers species

Also helpful in woodland identification is species phenology. In early spring deciduous trees are leafless. That allows not only for easy differentiation of forests, but makes analysis of the land structure easier. Identification of deciduous and coniferous trees is also easy with photos taken in autumn. For the purposes of forest identification, the most useful are pictures at a medium scale of up to 1:20 000.

Meadows

They are recognizable as dark-gray or dark-green areas sometimes having a cloudy texture. After grass harvesting a picture may be different with very strong visible strips, the effect of using harvesting equipment (Box 4.3).

Arable land

On aerial photos arable land is clear and brighter in colour, especially during summer months, and is characterized by a specific texture reflecting equipment use. Unfortunately, crop analysis is one of the biggest challenges in photointerpretation. It requires very detailed study of the brightness indicator of different crops on photos taken at particular times of the year.

Urban areas

Aerial photography is one of the most valuable sources of information about human settlement. In the case of villages, the most important properties are: shape, number of farms, building density or, for long-term documentation, also the rate of development as a result of interactions between a settlement and surrounding environment. In the case of towns these interactions are expressed by the arrangement of streets. History and functions of the city may be deduced from the distribution of buildings of different sizes and uses.

IMPORTANT PARAMETERS - QUANTIFICATION OF INFORMATION

How is the area of objects measured?

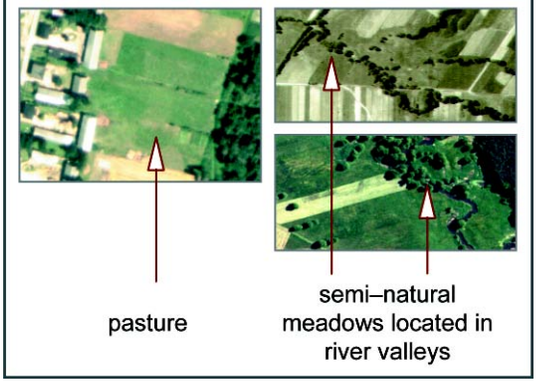
The area of objects may be measured directly on the picture if the topographic profile of the land is not very diversified. This is possible using a planimeter, but first the scale of the picture has to be calculated (Box 4.4). Because there are picture deformations, increasing from the centre to the edge, the most accurate estimations may be conducted only for objects situated in the centre.

How do you measure lengths?

On pictures of areas showing small elevation differences (up to 100 m) lengths may be measured directly with a ruler. If the lines are curved, the easiest way is to divide them into straight sections and to measure each separately. An alternative method is to use a curvometer. Very small objects require application of a Brinell magnifying glass or Brinell microscope.

BOX 4.3

How are meadows and pastures identified?



pasture

semi–natural meadows located in river valleys

How is patchiness estimated?

Patchiness is related to the average patch size (APS) in a chosen fragment of landscape:

$$APS = \frac{Sf}{n}$$

where Sf = area of the landscape fragment and n = number of patches.

To calculate the temporal changes of patchiness we need only to modify the equation:

$$\Delta APS = \frac{Sf}{n_1} - \frac{Sf}{n_2}$$

In this case n is the number of patches located on area, Sf, or patches with particular characteristics like woods, meadows, etc (Chmielewski, 2001).

How is ecotone density estimated?

Ecotone density is simply calculated as:

$$D = \frac{L}{S}$$

where: D = ecotone density, L = ecotone length and S = analyzed area.

Then the long-term change in density of transitional zones is calculated as:

$$\Delta D = \frac{100\left(\frac{L_1}{S} - \frac{L_2}{S}\right)}{\frac{L_2}{S}}$$

where ΔD = indicator of density change in transitional zones [%], L_1 = initial length of the transitional zones, L_2 = final length of transitional zones, and S = area of analyzed land (Chmielewski, 2001).

BOX 4.4
How is the scale of the picture determined?

Aerial photos should always be compared with maps of the area.

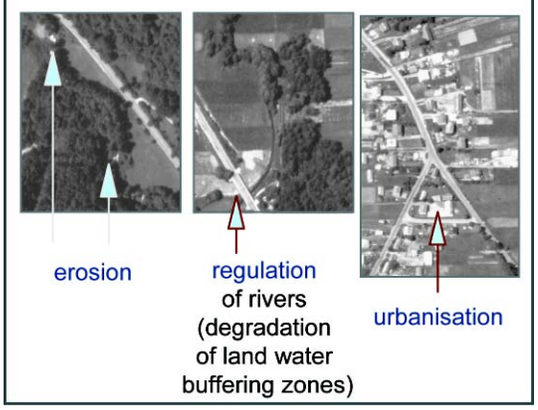
The following equation can be used:

$$\frac{l}{m_p} = \frac{l_p}{L_m \cdot m_m}$$

where:
l – length of the line on the picture (p) and on the map (m);
m – denominator of the scale of the picture (p) and on the map (m).

(Cieślewski et al., 1999)

BOX 4.5
How are threats identified?



What are the sources of data error?

Measurements based on photographs are not always accurate. There are the number of factors that are prone to error, which, if necessary, should be corrected mathematically.

The most important sources of inaccuracy are related to:

- photo slope (can be ignored if less than 3°);
- large elevation differences;
- lack of precision in identification of object borders;
- area deformation caused by cameras; and
- scale of the picture.

IDENTIFICATION OF ENVIRONMENTAL THREATS

At the landscape level there are several signs of loss of ecosystem resilience, which should be considered during analysis of photographs (Box 4.5). All of them are related to the potential for water and matter storage by landscape structures.

Regulation of rivers

Changes in riverbed shape, appearance of embankments and, what is even more spectacular, decline or disappearance of land-water plant buffering zones, indicate loss of connectedness between a river and its valley. It leads to high hydrological variability, decline of water levels and discharges, water shortages in agricultural areas, increases of chemicals leaking into surface and ground waters, and, finally, a decline of biodiversity.

Erosion

Areas undergoing erosion are identified as lighter patches situated along a river corridor. The indicator of bottom erosion is a deepening of the river valley and a tendency of the river to occupy its whole width.

Erosion may be natural, resulting from the geological and morphological structure of the catchment (e.g., loess areas) or human-induced. In the latter case, it usually reflects degradation of the vegetation cover and inappropriate distribution of different use areas. The cause of erosion may be located far from the place where it is observed.

Urbanization

The increase of the percentage of urban areas compared to natural and semi-natural areas is an indicator of possible water and environmental quality deterioration.

Analysis of the rate and direction of expansion of urban areas may provide additional information about potential threats to the environment and the necessary management counteractions.

Fragmentation

It is well known that medium land patchiness is the optimal one for sustainable landscape management.

Landscapes characterized by large, homogenous areas are a source of non-source pollution, erosion and, hence, water siltation. From a biological point of view, they also encourage the spread of diseases and biodiversity loss.

High patchiness of the landscape, especially when resulting from increased density of anthropogenic ecotones, disturbs chemical and water circulations in a catchment. It also does not provide stable conditions and space required by organisms for completing their life cycles.

APPLICATION OF GIS TECHNIQUES

Geographical Information System (GIS) is a program which has the capability of storing and analyzing digital maps and remote sensing images using different software components (Box 4.6).

GIS may operate easily at the single ecotone scale up to the largest catchment scale. It is used for:

- visualization and communication (distribution, properties, spatial relations between objects);
- to measure and inventory (e.g., how much of a resource is present); and
- analysis, prediction, modelling and decision making.

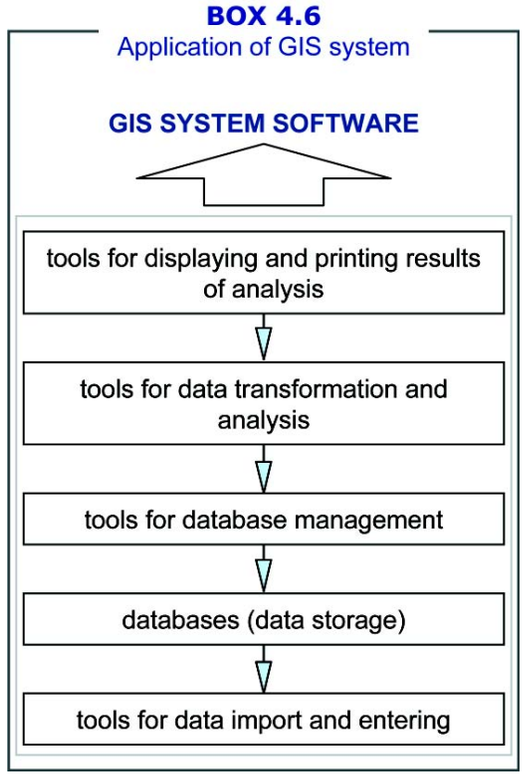
How does a GIS system operate?

Every natural phenomenon has a spatial and temporal dimension, which means it occurs at a given location and at a particular time. Natural objects (entities) are represented in GIS by:

- loation (typically expressed as: x-y, or eastings-northings, or latitude-longitude coordinates); and
- attributes (type of observation, counts, measured value, time, etc).

The location of an object is defined within a coordinate system, e.g., the Cartesian one (coordinates x, y - for a flat surface on map) and the latitude-longitude system (coordinates x,y - for the glo-

BOX 4.6
Application of GIS system



be and in GPS (satellite positioning systems). Data described in orthogonal cartographic coordinates make it possible to perform cartometric calculations of object length, area or volume. Attribute data can be a name, number, class value or qualitative description (verbal, pictorial or numerical). **Geometric representation** of an object in a given coordinate system is a **point**, **line**, or **surface**.

- **Point** does not have a topological dimension, but in GIS programs it can be displayed as a symbol (for example, sampling point, source of pollution).
- **Line** is a sequence of points (segment), in which we may distinguish start and end po ints and nodes at an intersection of lines. Lines can be additionally described by code. For instance, elevation of a contour line. Lines may be used to represent linear features like streams, roads, and boundaries.
- **Surface** represents two-dimensional objects (lake, forest). Three-dimensional objects are represented as a surface, which have an elevation attribute (for instance, a digital terrain model).

Data visualization - analysis

In computer graphics there are two data models to represent the geometry of objects - vector and raster.

- Lines written in **vector format** may be used to represent networks (for example, river system), connected lines may enclose polygons or areas that are homogenous, e.g., land unit;
- A **raster** is a regular matrix of elementary cells or pixels. The location of each pixel is determined by the size of the grid and the pixel size or resolution. Each pixel stores the attribute data for that particular location.

Many GIS programs use both types of data formats. Both raster and vector layers should be registered in the same coordinate system. During registration a relationship between raster coordinates (i - row, j - column) and cartographic coordinates (x - eastings, y - northings) are calculated. In hybrid graphics it is possible to perform conversions between data types called **vectorization or rasterization.**

The most common method of representing the complex nature of the real environment in computers is to use thematic layers.

Mathematical operations used for data processing can be performed on one or many thematic layers, including a third dimension written in a DTM. Spatial data analysis can include processing of object attributes and/or object geometry. Arithmetic operators (+, -, *, /, ^, sqr) are used in cartometric calculations (distance, area, volume, direction) and in processing object attributes. Relational operators (=, <, >, =<, >=, <>) are used for processing attributes and are useful for selecting certain objects according to given criteria. Logical operators (OR, AND, NOT, NOR) are used for finding objects belonging to many thematic layers according to specified conditions. Statistical operators are concerned mainly with attribute values. They are used to calculate, histograms, variance, distribution and attribute value correlations. More advanced methods are cluster analysis, semivariogram, and econometric functions.

In addition to simple operators we may perform more complicated analyses like map overlays (crossing) and proximity analysis (buffer zones, network functions). GIS is also a good environment for data preparation for external mathematical models and visualization of the modelling results.

DATA INTERPRETATION FOR ESTIMATION OF LANDSCAPE SENSITIVITY

The capacity of the landscape to absorb anthropogenic pressures may differ due to geomorphology, precipitation pattern, structure of plant cover, land use and its intensity, number of inhabitants, etc.

Although there is no precise recipe for optimal land use, one can easily identify symptoms of declining landscape resistance:

- land fragmentation;
- rapid development of urban areas;
- increased erosion;
- water siltation;
- decrease of forested areas, shrubs, tree lines; and
- degradation of land/water plant buffering zones and wetlands.

Identification of these processes and their quantification, together with more detailed studies including analyses of water chemistry and biota, may allow for the elaboration of effective landscape management strategies based on phytotechnological and ecohydrological approaches.

MAKE SURE TO CHECK THESE RESOURCES:

Guidelines: chapters 3.A-3.G, 4.A-4.E, 4.G

http://www.eman-rese.ca/eman/ecotools/protocols/terrestrial/vegetation/glossary.html

http://www.thewaterpage.com/aq_eco_july_01.htm

http://rst.gsfc.nasa.gov/Front/tofc.html

http://www.esri.com/software/arcview/index.html

4.C. HOW TO ASSESS SOIL CONTAMINATION

Polluted soils are a widespread issue and are problematic in terms of remediation. This is particularly true in the case of persistent pollutants (e.g., heavy metals and radionuclides), which are not amenable to natural attenuation. Soil remediation is expensive and often results in an alternative set of environmental considerations. As with all remediation efforts, a comprehensive understanding of the soil and the contamination are critical.

This chapter outlines major considerations in the design and implementation of a characterization program for contaminated land.

WHY SHOULD WE ANALYZE SOIL CONTAMINATION?

Soil pollution has to be analyzed in a broad context due to the potential for interactions among soil, ground water, surface water and air. Contaminated soil can affect all of these media, and through them, humans as well as other living organisms. The effects of soil pollution can be observed far away from the source, even hundreds of years after the polluting activities have ceased (Alloway, 1995).

LAND SENSITIVITY

Approaches to the assessment of land contamination differ, depending on the present or anticipated future land use. Fundamental questions include how the site will be used, who will use it and, „how clean is clean". The key issue is known as „land sensitivity", which is used to determine the maximum tolerable contamination levels when the land is used for a particular purpose. While overly simplified, the following land segregation categories may be used to illustrate this concept: (Box 4.7)

STANDARDS

Permissible legal values for acceptable soil contamination vary from country to country. In many cases, countries allow site-specific flexibility in the development of clean-up standards. In Poland, where standards are based on the Dutch and German approaches, three categories of land use are recognized with associated sets of standards: (Box 4.8)

Fig. 4.3
Collecting of soil samples
(photo: R. Kucharski)

BOX 4.7
Land sensitivity

SENSITIVITY	PURPOSE OF LAND USE
VERY SENSITIVE →	kindergartens, playgrounds, backyard gardens, farming areas
SENSITIVE →	houses, apartments
MODERATELY SENSITIVE →	offices, shops
LESS SENSITIVE →	industrial facilities, roads, parking lots

BOX 4.8
Recommended land uses

CATEGORY	PURPOSE OF LAND USE
A →	protected zones including national parks and water intake points
B →	farming, forest and residential areas
C →	industrial zones

TABLE 4.6
Soil contamination standards for some pollutants

CONTAMINANT	CAT. A	CAT. B	CAT. C
		DEPTH [m]	
		0,0 – 0,3	0,0 – 2,0
METALS			
Chromium	50	150	500
Zinc	100	300	1000
Cadmium	1	4	15
Lead	50	100	600
Mercury	0,5	2	30
HYDROCARBONS			
Gasoline (hydrocarbons C6-12)	1	1	500
Total aromatic hydrocarbons	0,1	0,1	200
Total polycyclic aromatic hydrocarbons	1	1	250
CHLORINATED HYDROCARBONS			
Aliphatic chlorinated hydrocarbons (total)	0,01	0,01	60
PCB	0,02	0,02	2
PESTICIDES			
DDT/DDE/DDD	0,0025	0,025	0,25

(source: Polish Standards, 2002)

Examples of permissible concentrations in Poland are shown in Table 4.6. Benchmark values are developed from basic toxicological literature.

From these permissible concentration values are calculated using risk assessment methods. The same approach is used when planning soil clean-up activities. Remedial activities are preceded by a baseline risk assessment that is compared to standards applicable to the anticipated site use. Target clean-up values then are developed that would be safe for a theoretical population using the remediated area under the assumed conditions. This procedure is carried out routinely to evaluate the need for, and extent of, remediation (US EPA, 1989).

THREATS TO THE ENVIRONMENT

In many cases, the most vulnerable segment of the population is young children, whose direct and indirect exposure to contaminated soil is magnified by their physically active lifestyle and various exposure-increasing habits (e.g., licking dirty hands and toys (Roper, 1991). Exposure scenarios that consider childhood exposure are often the major factors in determining site remediation needs.

Agricultural land pollution plays a crucial role in the exposure of local populations to heavy metals. The consumption of contaminated agricultural products can be a significant source of contaminant exposure. Humans take up most metals through the digestive tract after ingesting contaminated foodstuffs, especially vegetables. The major source of heavy metal contamination to plants comes from soil pollution (Kucharski et al., 1994).

Another important exposure scenario involves site excavation, either for routine activities or for remedial purposes. Such a scenario envisions workers in direct contact with subsurface soils.

BIOAVAILABILITY OF POLLUTANTS CONTAINED IN SOILS

Contaminants can exist in soils in a variety of chemical forms. These forms have varying uptake rates by living organisms (bioavailability). Bioavailability describes the ability of a specific chemical form to cross biological membranes (i.e., actually enter an organism). For example, very little of the metallic form of mercury is taken up following ingestion, while organic forms of mercury readily cross gastrointestinal membranes.

Bioavailability of soil contaminants influences the risk posed to living beings and, thus, the need for, and extent of, remediation required to insure the safety of exposed populations. In soils, bioavailability is influenced by physical, chemical and biological factors (including root, bacterial and fungal activities), as well as mechanisms of absorption into plants or animals (Brümmer et. al., 1986). Sequential chemical soil analysis is used to describe the speciation or bioavailability of metals. However, most legal regulations address only total concentrations of contaminants and do not take into consideration chemical speciation. However, this information can be used to evaluate the potential for adverse impacts from soil contamination on the food chain (Tessier et. al., 1979).

HOW IS SOIL CONTAMINATION ASSESSED?

What is the goal of site characterization?

The goal of site characterization is the development of a conceptual site model that will be used to determine the need for, develop and guide, site remediation. The data generated from the chemical analysis of soil samples is further processed using statistical and visualization (e.g., GIS) processes.

Careful planning and implementation of site characterization is vital to the effective clean-up of a contaminated site. The initial involvement of all members of the remedial team (e.g., field personnel, soil scientists, chemists, statisticians and toxicologists) when planning site characterization activities will streamline the process, reduce the need for additional characterization efforts and result in more effective remediation.

How to collect representative samples

A sampling grid typically is used to determine the spatial variation of contaminants at a site. The sampling scheme needs to determine the extent (in both area and depth) and spatial variability of contaminants at the site, initially and during remedial activities. The number and location of samples will be site specific. Soil sampling patterns depend on a number of factors including site history, expected data needs and planned remedial strategy. There are a number of possible patterns for site characterization, e.g., circular grid, random, stratified, zigzag, transverse sampling and systematic (Box 4.9, ISO/CD/10381-5). For initial characterization of soil contamination (i.e., screening), samples are obtained using a sampling grid. An example of a field data sheet is shown in Appendix 1. which facilitates the systematic sampling and handling of samples (US EPA, 1989a)

How deep should we sample?

The depth of samples is important to consider and should be based, in part, on the history of the site. It is important to remember the old adage that „one will not find that which is not sought". Initial soil sampling often screens samples from several depth ranges (e.g., 0-15, 15-30, 30-45 cm). The top 30 centimeters of soil generally is considered when evaluating the threat posed to living organisms by contaminated soil (see chapter 9A). In some cases, e.g., construction purposes, where deeper layers of the ground will be exposed, a different and more extensive set of considerations will need to be applied.

Once the depth of contamination is known, further sampling can be concentrated in that depth range. Soil samples should be collected using equipment that is compatible with the physical and chemical needs of the sampling plan (e.g., disturbed vs. undisturbed, spot vs. composite samples). Plastic samplers or tubes generally are used for samples intended for metal analysis, while metal

samplers generally are used for samples intended for organic analysis. The most commonly used equipment for collection of spot samples is a split tube soil sampler (ISO/CD 10381). Contaminant presence is quantified using routine laboratory methods (ISO/DIS 11464).

What parameters should be determined in a soil sample?

Soil is a complex media and a number of variables exist which may influence the fate of anthropogenic substances introduced as contaminants.

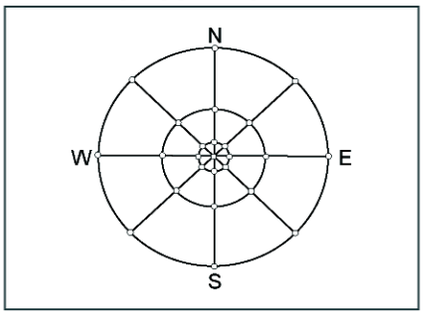
Fig. 4.4
Mechanical auger
Forestry Suppliers, Inc.
Catalog 51, 2000-2001

Some basic factors can be isolated which are crucial to soil susceptibility. Basic soil parameters can be grouped into four groups (Korcz et al., 2002):

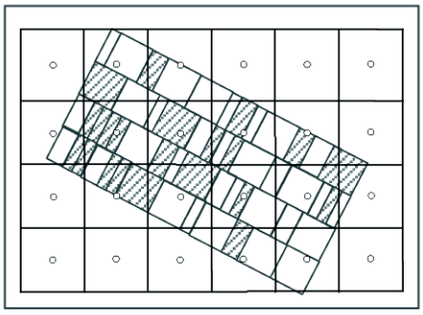
- **mass transport characteristic** (soil texture, unsaturated hydraulic conductivity, dispersivity, moisture content/tension, bulk density, permeability, infiltration rate, soil layering);
- **soil reaction characteristics** that describe soil-contaminant reactions (soil-water partition coefficient, cation exchange capacity, acidity, redox, soil biota, soil nutrients, contaminant abiotic/biotic degradation rates, soil mineralogy);
- **contaminant properties** - solubility in water, dielectric constant, diffusion coefficient, organic carbon partition coefficient, soil/water partition coefficient, Henry's constant, molecular weight, vapour pressure, density, chemistry of water extracts; and
- **soil engineering characteristics** and properties (erodibility, depth to ground water, thickness of unsaturated and saturated zones, depth and volume of contaminated soil, bearing capacity).

BOX 4.9.
Patterns for site characterization

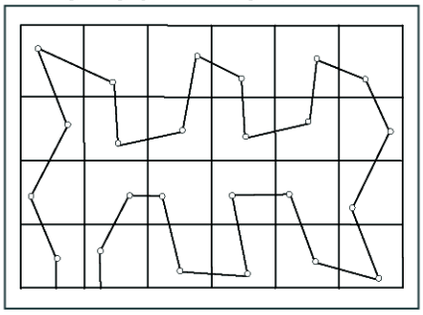
CIRCULAR GRID



RANDOM SAMPLING WITHOUT GRID



ZIGZAG SAMPLING PATTERN



To obtain basic information on soil properties, the following analyses are routinely made:

- soil type, texture;
- conductivity;
- pH;
- content of organic matter;
- concentration of pollutants in question;
- fertility (vegetative capability);
- depth to ground water;
- surface runoff; and
- permeability.

Photo 4.5
Various types of soil sampling equipment
Forestry Suppliers, Inc. Catalog 51, 2000-2001

MAKE SURE TO CHECK THESE RESOURCES:

Guidelines: chapter 5.B

5.A. CAN GROUND WATER INFLUENCE SURFACE WATER QUALITY?

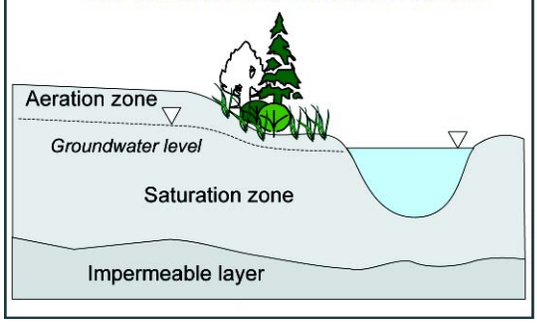
Groundwater, especially in catchments with intensive agriculture and grazing, may transport a considerable amount of dissolved pollution to fresh waters. During the growing period, pollution can be effectively diminished by application of phytotechnological methods. However, their application requires preliminary studies. The objective of this chapter is to introduce methods for analyzing groundwater movement and chemical composition in order to define the risk related to the transportation of contaminants from a catchment to lakes, rivers and reservoirs.

Fig. 5.1
Sampling ground water at the Pilica River - an ecohydrology and phytotechnology Demonstration Site in Poland (photo: I. Wagner-Lotkowska)

WHAT IS GROUND WATER?

The term ground water describes water present in the saturation zone that is separated from the earth's surface by a permeable zone of aeration. Ground water is delimited from belowby an impermeable basement (impervious layer), from above by a free water table and aeration zone (Box 5.1).

BOX 5.1
The aeration and saturation zones



A groundwater table usually has a slope called the **hydraulic gradient**.

Ground water is susceptible to changes of temperature (up to 20 m below the ground surface), water table level fluctuations and chemical composition. Precipitation influences the groundwater level, but the deeper below ground level the water table is, the more delayed are its changes. Ground waters are interconnected with surface waters in two ways:

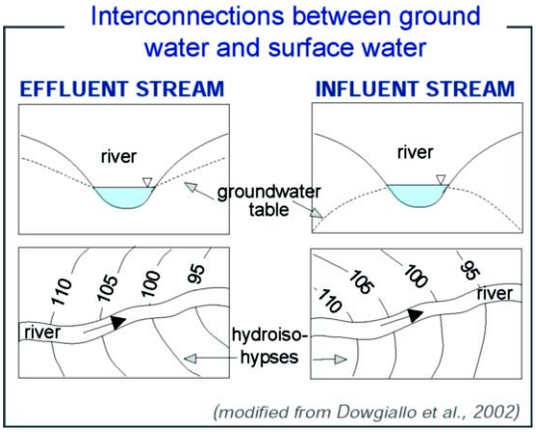
- streams supplied by ground water, or effluent streams; and
- groundwater supplied by streams, or influent streams (Box 5.2).

Identification of the stream character can be done using hydroisochip diagrams:

- ground water drained by a riverwhen the groundwater table falls towards a stream and
- infiltrating stream, when the underground water table reaches the highest level near a stream bank and falls towards a valley.

The characteristics of interaction between a stream and underground water can change both spatially and temporarily: the stream can be an effluent and change into an influent or vice versa.

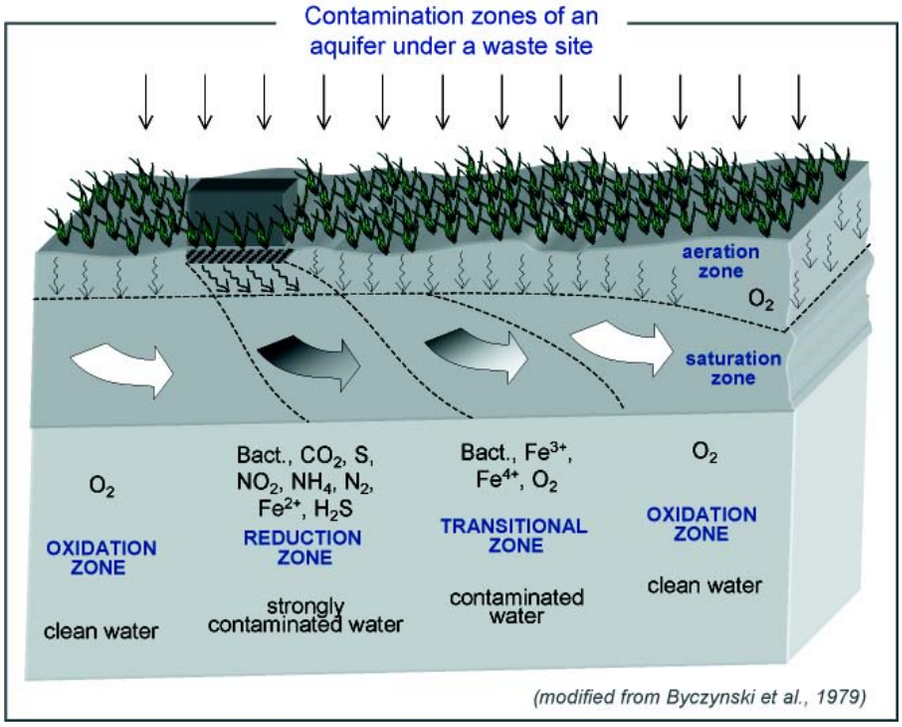
BOX 5.2
Interconnections between ground water and surface water



GROUNDWATER SUPPLY SOURCES

Supply of underground water is provided by so called **effective infiltration**. Precipitation infiltrates to deeper strata (Box 5.3), but part of this water is kept in the aeration zone and used by plants.

BOX 5.3
Contamination zones of an aquifer under a waste site



The other part of the water penetrates deeper into the **saturation zone**. Water supplying ground water causes its level to rise and is called effective infiltration. The volume of infiltration depends on the quantity and intensity of precipitation, soil type and its initial humidity, plant cover etc. Shortages of precipitation and ground with low humidity may result in the storage of the all rainfall in the unsaturated zone.

GROUNDWATER CHEMISTRY

Chemical composition of ground water is influenced by:

- precipitation;
- chemical composition of the soil;
- period of water cycling;
- relief and vegetation;
- land use and anthropogenic activity

GROUNDWATER CONTAMINATION

Underground water is subjected to pollution connected with human activity, origining from agriculture, farming and settlements.

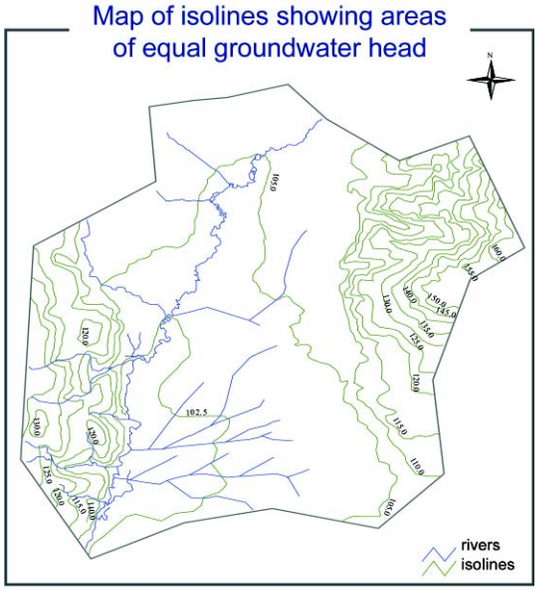
Pollution can manifest itself as follows:

- increased concentrations of ionscommonly appearing in groundwater, e.g., NO_3^-, NO_2^-, PO_4^{3-}, K^+, Na^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, SO_4^{2-}, Cl^-;
- appearance of man-made organic substances (pesticides and products of their degradation);
- increased mineral matter, conductivity, solid residue, and water hardness; and
- increased oxidation rate, BOD and bacterial contamination (see Guideline chapter 5).

All pollutants while being dispersed in the ground undergo alterations. The following factors influence the potential threat of pollutants:

- presence and thickness of impermeable layers above aquifers;
- thickness and type of soil cover;
- depth of aquifers; and
- self-purification processes of infiltrating water.

BOX 5.4
Map of isolines showing areas of equal groundwater head



COLLECTION AND ANALYSIS OF LANDSCAPE INFORMATION

The first step, which should be done to assess pollutant infiltration processes from the surface into ground water supplying a stream, is collection of basic information such as:

- **maps**: topographic, soil, hydrographic, and geological from geological and hydrological archives for:
  - average annual and monthly precipitation, preferably for the last 25 years;
  - average annual snow precipitation and duration period as well dates of the appearance and disappearance of the snowcover;
  - average multi-annual duration and start dates of the growing season; and
  - average annual and monthly run-off (in mm).
- **statistical data** (use of fertilizers, pesticides, doses and usage periods, land use structure):
  - history of land use, preferably for last 100 years;
  - recent land-use patterns (forests, arable lands, grasslands, urban areas, waters); and
  - characteristics of anthropogenic activity in the catchment and its nearest vicinity: industrial plants, rail routes and roads, intensive agriculture and farming, forestry, touristic and recreational pressures, changes in water budget, etc.

Field studies and sample collection

The first step in field studies should be identification of „hot spots" in the catchment, which can potentially influence groundwater quality:

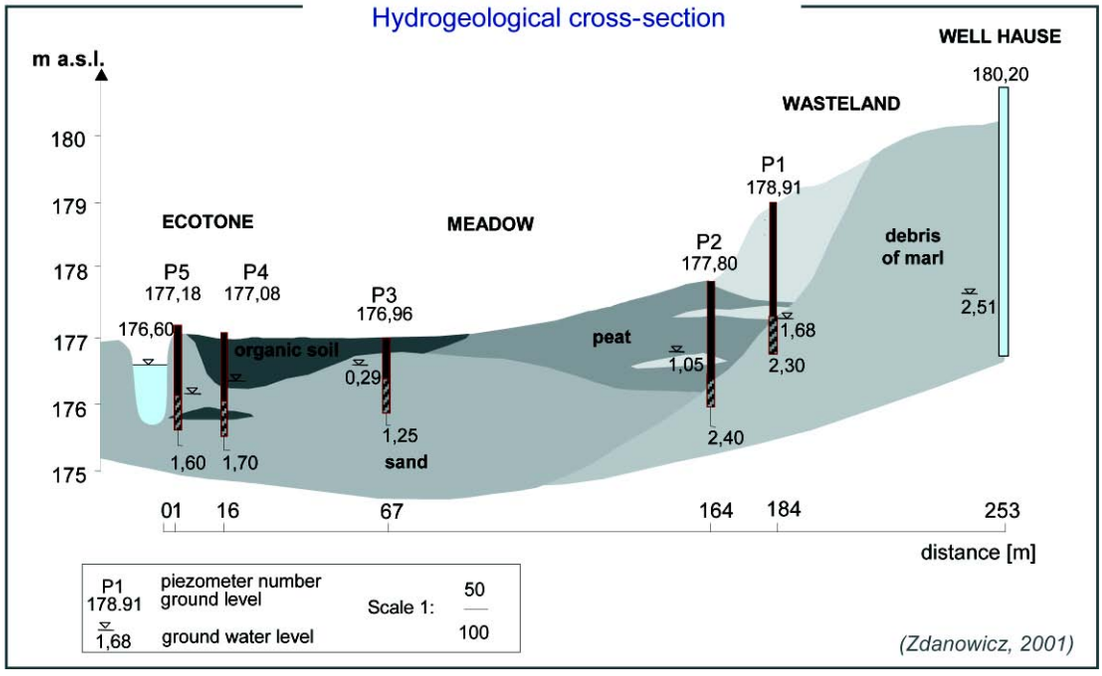
- localized point sources of pollution (village, dumps, pesticides store houses, fertilizers, chemicals, cesspools, filling stations, etc.; and
- identification of non-point source pollution (fertilizers used in agriculture).

Data on water quality and transfer of contaminants with groundwater movement to surface water can be obtained from piezometers. There are installed in transects located along the gradient of groundwater flow from a pollution source toward a stream (Box 5.4).

All piezometers in a transect should be denivelated in a floodplain according to the national geonet using a tachymeter in order to obtain precise data from each piezometer (Box 5.5).

BOX 5.5
Nivelator

BOX 5.6
Hydrogeological cross-section



Simultaneously with drilling holes for inserting piezometers, samples should be taken for determination of soil granular composition. This information is used to determine the filtration coefficient (k) using, e.g., the USB method. On the basis of these results, the hydrogeological section pattern for a given transect can be created. (Box 5.6).

Groundwater Measurements

Grounwater level can be measured with various methods. The most frequently used and simplest tool to determine groundwater levels is a measuring tape that is inserted into a piezometer.

Sampling

To obtain water samples from a piezometer, pumps and samplers are used. The following steps should be followed:

- Determine groundwater level;
- pump out a minimum of three volumes of the water column flowing into a piezometer;
- sample water for chemical analysis.

Field measurement procedures:

- record water temperature - preferably by digital or liquid therometre exact to 0.1°C;
- record pH with a pH-metre; and
- record conductivity with a conducivity me tre;
- water sampling for chemical analyses in the laboratory: store water samples in propylene containers.
  - anion samples - capacity of 250 cm^3; the container should be filled to the top without leaving a headspace;
  - cation samples - capacity of 60-125 cm^3, preserved with hydrochloric acid. Add 5 cm^3 HCl (1:1) for each 100 cm^3 of water sample. Samples should be filtred through a membrane or glass-fibre filter.

Estimation of groundwater flow rate using the empirical method

True flow velocity can be calculated from the expression:

$$U = \frac{V}{\mu} [ms^{-1}]$$

where:

μ - effective porosity (read from a table)

v - apparent velocity calculated using the expression:

$$V=ki$$

where:
k - filtration coefficient [m d^{-1}],
i - hydraulic gradient [m], calculated using the expression:

$$i=\frac{\Delta h}{l}$$

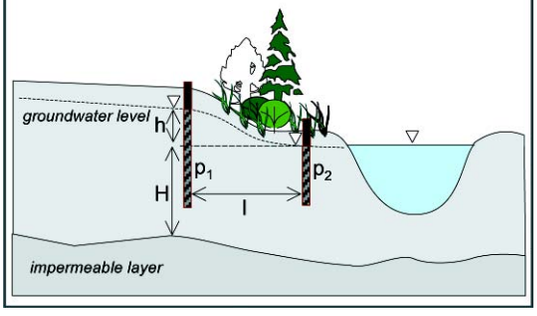
where:
Δh - difference between grounwater table levels of two piezometers [m],
l - distance between the piezometers.
(Box 5.7)

Sampling frequency

Water samples should be taken twice a week or monthly.

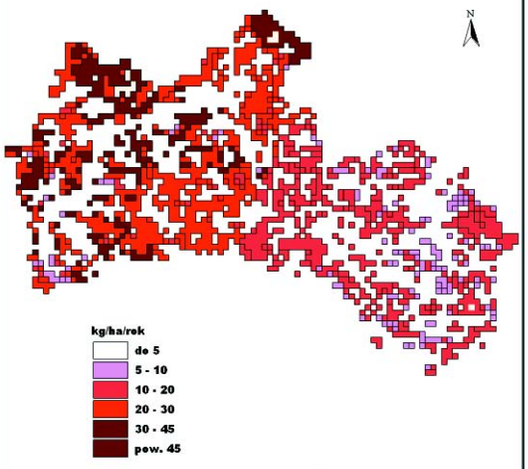
BOX 5.7

Example of groundwater flow rate calculation: index method



BOX 5.8

Example application of a GIS programme (Arc Info, Arc View). Map of nitrogen content in ground water



(Zdanowicz, in press)

INTERPRETATION AND VERIFICATION OF RESULTS

Data analysis starts by gathering information from the different measurements, tabulating it and then preparing graphs and diagrams using appropriate software (e.g., Excel, Statistica, Grafer, Corel).
Results of landscape studies are presented in the form of maps using GIS: Arc Info, Arc View, Map Info or other types of software (Box 5.8).
Prediction scenarios of groundwater quality changes and analysis and timing of pollutant flows from ground water to surface water can be estimated on the basis of mathematical modelling using models of pollutant transport, e.g., FLOTRANS, MOTFLOW, MT3D, etc.

MAKE SURE TO CHECK THESE RESOURCES:

Guidelines: chapters 1.E, 3.A-3.G, 9.A-9.C
www.scisoftware.com
www.goldensoftware.com

5.B. HOW TO ASSESS THE EFFICIENCY OF ECOTONES IN NUTRIENT REMOVAL

Ecotone zone is the transition between two different ecosystems. From the point of view of water protection and restoration of freshwater ecosystems, a special role is attributed to land /water ecotones (riparian zones). They regulate the exchange and spreading rates of chemical compounds between terrestrial and water systems therfore they are buffers or filters in landscapes.The objective of this chapter is to introduce some basic information related to estimation of the role of ecotones in regulation of nutrient retention in a land-water transitional zone.

NUTRIENT MOVEMENT

Depending on the dominant type of land use, nutrients move from terrestrial ecosystems into ground and surface waters at different levels.

The most important nutrient sources are cereal monocultures, although arable lands in general should be considered as a cause of water eutrophication (Table 5.1).

TABLE 5.1
Range of total and dissolved phosphorus loss from land used for different purposes

LAND USE	total phosphorus	dissolved phosphorus
arable land	0,04-6,3	0-0,13
forested land	0,02-1,0	0,02-0,08
pastures	1,1-5,6	0,4-2,4
cereal cultures	0,1-67,0	-
urban areas	0,92-56,0	0,9-2,0

(Hilbricht-Ilkowska, 1995)

The role of intense land use and increasing fertilizer application in worsening water quality is exaggerated by the loss of buffering zones, especially riparian ecotones that have been transformed into pastures or arable lands.

WHAT IS THE ROLE OF ECOTONES?

There are many different types of riparian ecotones: swamp forests, bank vegetation, meadows, littoral zones, marshes, floating mats, oxbow lakes, etc. Their common feature is occasional flooding. The water regime modifies the rates of aerobic and anaerobic biochemical processes and hence seasonal releases and removal of phosphorus and nitrogen.

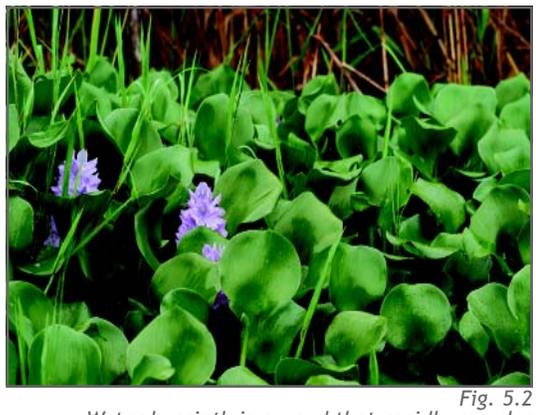


Fig. 5.2
Water hyacinth is a weed that rapidly recycles nutrients in aquatic ecosystems (photo: V. Santiago-Fandino)

Although the role of riparian vegetation is pronounced in regulating biochemical cycling, it may be much broader, including:

- preventing banks from being eroded;
- regulating water temperature and light penetration to a river bed;
- therefore also regulating primary production in streams and reservoirs; and
- creation of habitats for fauna.

CONNECTIVITY BETWEEN STREAMS AND ADJACENT SYSTEMS

The influence of ecotone vegetation on water quality is possible only when the connection between terrestrial and water ecosystems is maintained. The basis for this connection is water circulation, therefore, the role of ecotones is significant only along unregulated river courses and natural shores of reservoirs.

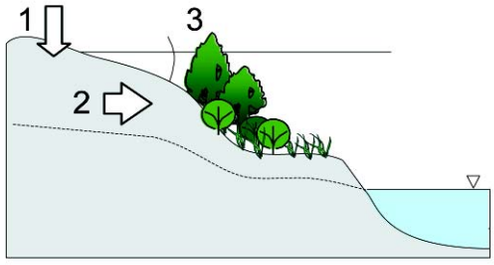
Under natural conditions, the influence of plant buffering zones on biochemical processes in fresh waters may vary from place to place according to the local geomorphology, soil type, moisture, interactions between plants and other organisms, etc (Box 5.9).

In the case of rivers it is also necessary to consider role changes of ecotones along a river course caused by changes in the water volume/length relationship (Box 5.10).

BOX 5.9

Three main groups of factors which influence the efficiency of ecotone zones in nutrient removal

1. nutrient supply ratio – depending on the type and intensity of land use;
2. ground structure and soil characteristics as they decide about sorption capacity of a zone; and
3. catchment incline (for details see the MANAGEMENT part of the Manual).



NATURAL PROCESSES INVOLVED IN NUTRIENT REMOVAL

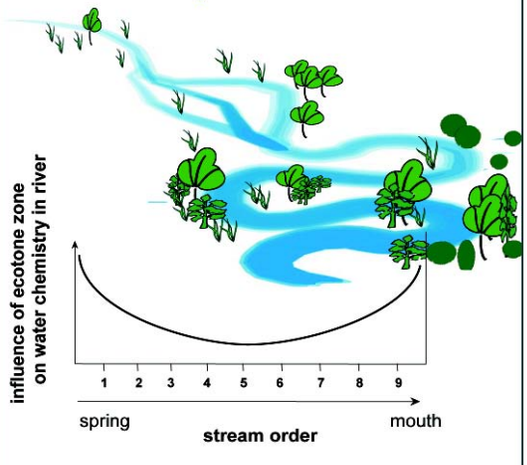
Biochemical processes

There are two groups of processes responsible for nutrient retention and transformation:

- those occurring in **aerobic layers** - precipitation and sorption of P on clays (due to presence of Al, Ca, Fe ions), nitrification of N; and
- in **anaerobic layers** - release of P, fixation and denitrification of N (at oxygen concentrations below 4 mg L^{-1}).

BOX 5.10

Changes of ecotone influence on biochemical processes along a river course



(modified from Schiemer, 1999; Naiman, Decamps & Fournier 1989)

As the presence of aerobic and anaerobic conditions is dependent on water level, it is of great importance to preserve the hydrological dynamics (rate and timing of events) in a landscape typical for dominating ecotone communities.

Role of vegetation

The role of riparian vegetation in maintaining the resilience of freshwater ecosystems is based on:

- increase of infiltration of surface flow;
- decrease of surface flow velocity due to greater coarseness of groundcover vegetation;
- enhanced sedimentation; and
- nutrient retention in soil and plant tissues.

Naturally occurring riparian communities in temperate climates: alder forests (*Alnetea glutinosae*), mixed ash and alder forests (*Querco - Fagetea*), wet meadows (*Molinio - Arrhenatheretea*) and rushes (*Phragmitetea*).

They may appear separately or form successive zones significantly reducing nutrient concentrations in ground waters and diminishing surface flow from agricultural areas (Klosowski, 1993).

Zonation and species composition, together with hydrological and soil conditions, determine the physical structure of riparian habitats. This is worth underlining because plants themselves accumulate only 10-50% of nutrients passing through the buffering zone (mostly during the growing season). The remaining pollutants are retained by other ecotone components (Box 5.11).

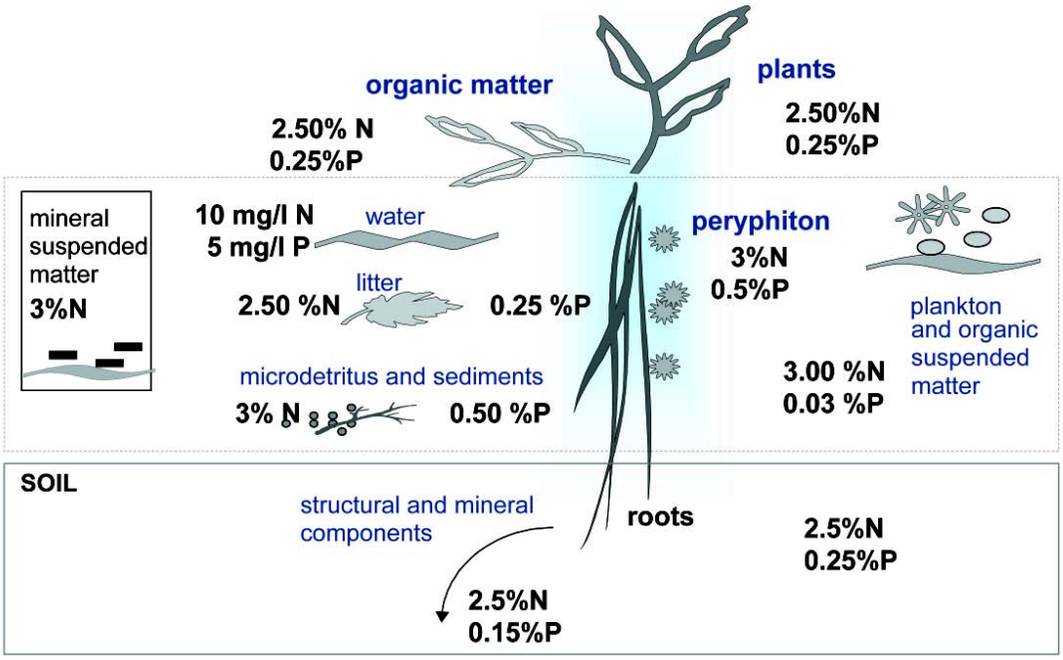
Ecotone efficiency

According to Petersen et al. (1992), the efficiency of wide ecotone zones (19-50 m) may reach even 78-98% removal for N in surface waters and 68-100% in ground waters. Other authors estimate reduction efficiency at a level of 50-90% for nitrogen and 25-98% for phosphorus (in ground water) depending on the initial concentrations, width of buffering zone, soil type and according to Intermediate Complexity Concept (see chapter 11.C) the complexity of the ecotone structure (Peterjohn & Corella, 1984; Verhoeven et al., 1990).

It has also been found that the most intense reduction occurs within the first 10 m of an ecotone zone (Box 5.12).

BOX 5.11

Mechanisms and elements involved in nutrient retention in buffering zones covered with reeds (*Phragmites australis*)



(modified from Kadlec & Knight,1996)

HOW TO ASSESS THE EFFICIENCY OF ECOTONES IN NUTRIENT REMOVAL

Assessment of nutrient removal by ecotone zones requires:

- detailed geomorphology and aquifer analysis prior to the setting of sampling points;
- assessment of plant composition and zonation and preparation of maps;
- determination of the dominant type of land use in the neighbouring area; and
- installation of piezometer nets in transects across an ecotone zone - the distribution should reflect the plant zone distribution, including the area being considered as a pollution source and the recipient fresh water (Box 5.13);

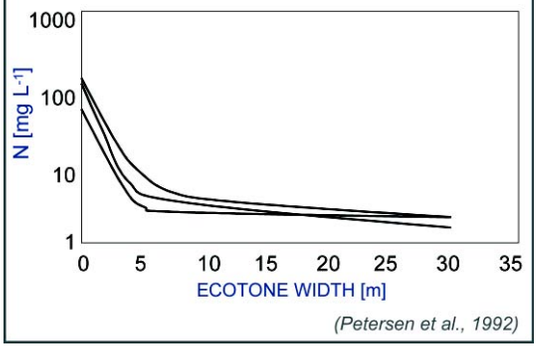
Piezometer installation should be carried out in the season when the water level is the lowest (June-July in temperate climates). The phosphorus and nitrogen loads transported into a reservoir via ground water are calculated on the basis of underground inflow (L s^{-1} km^2) and concentration of nutrients (mg L^{-1}).

The physical-chemical analysis of water samples should include: pH, temperature, conductivity, oxygen concentration, dissolved forms of phosphorus and nitrogen - PO_4-P, NO_2-N, NO_3-N, and NH_4-N, and total phosphorus and nitrogen concentrations.

- piezometers should be installed in drilled holes reaching the first attainable water layer.
- samples of ground water for physical-chemical analysis are collected after measurement

BOX 5.12

Pattern of nutrient reduction in water passing an ecotone zone



(Petersen et al., 1992)

TABLE 5.2
Data interpretation

temperature	temperature affects the solubility of many chemical compounds and can therefore influence the effect of pollutants on aquatic life. Increased temperatures elevate metabolic oxygen demand
pH	high pH values tend to facilitate solubilization of ammonia, heavy metals and salts. Lethal effects of pH on aquatic life occur below pH 4.5 and above pH 9.5
dissolved oxygen	dissolved oxygen affects the solubility and availability of nutrients, and therefore the productivity of aquatic ecosystems. Low levels of dissolved oxygen are the result of nutrient releases
conductivity	conductivity is useful for estimating the total ion concentration in water and may be used as an alternative measure of dissolved solids. It is often possible to establish a correlation between conductivity and dissolved solids for a specific body of water [dissolved solids = conductivity x 0.55 to 0.9 (usually 0.7)]
PO_4-P	this form of phosphorus is the most readily available for uptake during photosynthesis.
NO_2-N	since nitrite is also a source of nutrients for plants its presence encourages plant proliferation. Nitrite is toxic to aquatic life at relatively low concentrations
NO_3-N	nitrate is the primary form of nitrogen used by plants as a nutrient to stimulate growth. Excessive amounts of nitrogen may result in phytoplankton or macrophyte proliferations
NH_4-N	excess ammonia contributes to eutrophication of water bodies. This results in prolific algal growths that have deleterious impacts on other aquatic life, drinking water supplies, and recreation. Ammonia at high concentrations is toxic to aquatic life
total phosphorus	phosphorus is the most limiting nutrient, its input to freshwater systems can cause extreme proliferations of algal growth
total nitrogen	organic nitrogen is not immediately available for biological activity

of the water level inside the piezometers (e.g., by using a depth indicator bound to a tape measure) and movement of the water (see chapter 5.A).

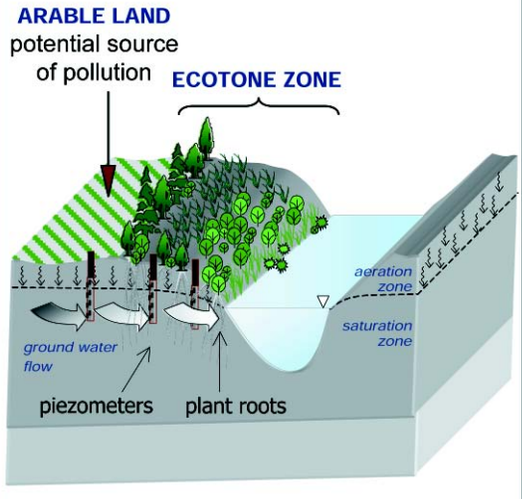
- during the drilling and installation of piezometers, soil samples should be taken in order to assess the characteristics of a soil profile and to estimate the filtration coefficient of subsequent layers.

DYNAMICS OF NUTRIENT UPTAKE AND REMOVAL - CONCLUDING REMARKS

The dynamics of nutrient uptake by natural ecotone zones is determined by:

- **Land geomorphology** - in upland and mountain areas riparian ecotones are poorly developed and the incline of the catchment promotes rapid surface flow. Therefore, emphasis should be put on proper land management and development of biogeochemical barriers over a whole catchment area.

BOX 5.13
Installation of piezometers



Collected water samples have to reflect the vegetation structure of an ecotone and show the initial concentration of nutrients in ground water (the first sample should be located close to a pollution source).

- **Nutrient load** - it was found that P removal rate exceeds 50% only when loading rates were lower than 50kg ha^{-1} $year^{-1}$.
- **Season** - it was estimated that in temperate zones vegetation works as a trap for nutrients for 9 months on average; effectiveness for N-trapping is higher and prolonged due to high microbial involvement in transformation processes.
- **Plant composition** - herbaceous vegetation is more active in nutrient uptake and accu mulation due to dynamic growth, while trees and shrubs block nutrients for longer periods and require less conservation.

Finally, it has to be stressed that natural ecotones and riparian wetlands in many cases play not only the role of nutrient barriers, but also transformers - they import inorganic forms of nutrients and export organic ones and also buffer and smooth nutrient pulses.

MAKE SURE TO CHECK THESE RESOURCES:

Guidelines: chapters 5.D-5.E
http://www.thewaterpage.com/aq_eco_groundw.htm
http://srmwww.gov.bc.ca/risc/pubs/aquatic/design/index.htm
http://www.thewaterpage.com/aq_eco_july_01.htm

5.C. HOW TO ESTIMATE EFFECTS OF RIPARIAN AREAS ANS FLODPLAINS ON WATER QUALITY AND QUANTITY?

Riparian areas are natural elements of each stream and river. They can be defined as an ecotone, or an extended system of ecotones and riparian areas located along a water body. However, usually their structure and role is more complex. Restoration and management of flooded areas is of crucial importance for proper functioning of river systems as well as the water bodies located downstream. The objective of this chapter is to present methods for identification of flooded areas and to assess their potential role in controlling the quantity and improving quality of water and the environment.

Fig. 5.3
Floodplain of the Pilica River in central Poland, a lowland river (photo: I. Wagner-Lotkowska)

WHAT ARE THE ELEMENTS OF A RIPARIAN AREA?

Riparian areas are complex systems that provide optimum habitat and food for stream communities. They are characterized by floral and faunal communities distinct from surrounding upland areas.

Generally, the following elements can be distinguished in riparian area:

- **swamp areas,** directly adjacent to the stream or river;
- **transitional swamp areas;**
- **ecological corridors**;
- areas covered with **organic soils**;
- areas of seasonally **high groundwater level** (0,6 m below the ground level);
- **hill slopes**, with slopes greater than 15 %, directly enclosing a stream or river;
- areas **flooded with a 100-year flood**, i.e., a flood with a 1% probability of recurring; and
- **buffering zones**.

Riparian may contain also such features as backswamps, delta plains, and oxbow lakes.

WHAT IS A FLOODPLAINS?

A floodplain is a flat area located alongside a stream or river channel that is inundated during high river discharges. Floodplains are formed by the deposition of sediments during periodic floods.

Floodplains are designated by flood frequency that is large enough to cover them. For example, a 10-year floodplain will be covered by a 10-year return-flood and the 100-year floodplain by a 100-year return-flood. This means floods that will reoccur with probabilities equal to 10% and 1%, respectively.

WHY SHOULD WE DESIGN AND PROTECT FLOODED AREAS?

Flooded areas play a variety of roles in regulating the quality and quantity of rivers, as well as that of reservoirs and lakes located downstream. To a great extent they may regulate the self-sustaining potential of a river ecosystem and increase it's absorbing capacity against such threats as landscape degradation and consequential increases of diffuse pollution and flood risk. Properly managed floodplains are responsible for:

- **stabilization of river discharge**, mitigation of the effects of **floods and droughts**;
- providing a framework for biogeochemical processes taking part at land-water interface zones and enhancing matter retention and self-purification of a river; and
- providing a number of transitional land-water **habitats** and supporting development of **biodiversity** in an area.

Floodplains are crucial for flood protection and discharge stabilization in lowland rivers;
Floodplains enhance self-purification of a river and are sinks for dissolved pollutants and nutrients as well as suspended solids transported by rivers during floods.

HOW TO IDENTIFY A FLOODED ZONE

Recognition

Depending on the scale of a river, the first step for identifying flooded areas and estimating their potential for water and pollutant retention, should be an analysis of maps and aerial photographs of a river corridor and adjacent areas located along it's channel (see chapter 4.B). If possible, it should be supported with direct observations in the field.

Data needed

The following information can be of importance for the preliminary work:

- **topographic and geological** characteristics of an area with special emphasis on the land located along a river;
- **hydrological characteristics** of a river and adjacent areas, including discharges of a river, distribution and density of other fresh water bodies and groundwater levels, if po ssible; and
- existing information about **land use and development of an area**.

The following materials, if available, can be useful for estimating the extent of a flooded area and their potential role for flood mitigation and water quality improvement:

- **maps of the area**
  - topographic maps - 1:10 000 scale (riverbed and adjacent floodplain areas);
  - topographic maps - 1:25 000 scale (floodplain and adjacent catchment);
  - topographic maps - 1:100 000 scale (catchment area for general overview of landscape use and structure); and
  - soil maps - 1: 5 000 scale.
- **hydrological and meteorological data**
  - yearly distribution of precipitation, snow cover, air temperature, potential evapotranspiration, in order to calculated **mesoscale** water circulation in the catchment; and
  - discharges with given probability of exceeding calculated values on the basis of a long term series of maximum discharges from gauged basins or determined by empirical methods in case of ungauged ba-sins.
- **hydraulics data** - roughness coefficient determined on the basis of inventory, land use maps, aerial photofraphs, and literature
- **geodesy data**
  - river cross-sections; and
  - cross-sections of existing hydrotechnical infrastructure.
- **others**
  - photographs of a river, river corridor and floodplains;
  - aerial photographs of potentially flooded zones; and
  - video documentation of historical floods, if available.

FIELD STUDIES AND MEASUREMENTS

To identify and assess the ecological potential of flooded zones for retention of water and matter, it is also necessary to carry out additional field measurements.

The basic information to be collected should cover the following:

- **characteristics of a riverbed** with detailed information about **hydrotechnical construction** and infrastructure; and
- detailed information about the **ecological value of an area**.

Characteristics of a riverbed

Special attention should be given to the natural structure of a riverbed. The following parameters should be listed for river sections of interest for potential management of flooded areas:

- distribution, length and characteristics of **natural river sections**;
- distribution, length and characteristics of **regulated and canalized** river sections;
- width, depth and **cross sections** of a river in all characteristic areas, with special emphasis on the section where flooded zones are to be restored and adjacent upstream and downstream floodplain sections;
- **cover and stability of a riverbed grain- size distribution**; and
- existing and planned **hydrotechnical constructions** along a river.

Appropriate consideration should be given to bridges, roads, human settlements and other infrastructures. The following aspects should be taken into account:

- **potential risk of floods** for human populations and infrastructure;
- **potential impact** of infrastructure **on water flow** (especially bridges, dams); and
- **potential impact** of infrastructure on water quality (especially location of sewage treat ment plants, tanks, industry etc. in areas po tentially flooded).

ECOLOGICAL VALUE OF AN AREA

In order to establish flooded areas to be restored, ecological studies should encompass the three following biotopes:

- water biotopes;
- land-water interface (flooded) biotopes; and
- land biotopes.

This assessment allows for the evaluation of the ecological value of particular biotopes. It also presents estimates of ability of flooded areas to maintain ecohydrological processes of importance for water and nutrient retention.

Assessments of biotopes can be conducted using either qualitative or experimental assessment methods. There are many methods for preparing ecological inventories. The choice depends on the target of investigation and type of development of an area and also on regional preferences and type of a river (mountain or lowland). In- spite of many determination techniques for this, they are subject to bias by a researcher's point of view and that is why experience is required. LÕLFA/ LWA (1985) is an example of the criteria that can be taken into account for assessment of all biotopes.

Ecological and landscape values of rivers are determined on the basis of an analysis that takes into account the following criteria:

- river morphology;
- hydrological characteristics;
- physical and chemical characteristics of water;
- river bed afforestations, water vegetation and water course scarp vegetation;
- biodiversity of biotopes, vegetation cover and distribution of native plant communities of a floodplain;
- river valley land use; and
- particular natural value of a valley.

Such analysis leads to determination of the natural water course category.

ASSESSMENT OF VEGETATION COVER

It is necessary to estimate vegetation cover of floodplain areas in order to estimate floodplain potential for assimilation of nutrients by vegetation biomass. The vegetation cover can be indicated on maps by using a GPS system (see chapter 4.B). It is recommended to identify the native plant communities and their ability for nutrient retention and biomass production under various hydrological conditions (groundwater level, timing of flooding).

ELABORATION OF A DIGITAL FLOODPLAIN MODEL

In order to identify an floodplain area for restoration, a digital terrain model (DTM) should be constructed. The model can be made on the basis of information from collected maps. In some cases, additional denivelation of a floodplain may be necessary.

Denivelation of a floodplain can be based on a network of altitude points created from irregular networks (TIN) by tachymeter measurements. On the basis of the collected data, the following information can be generated:

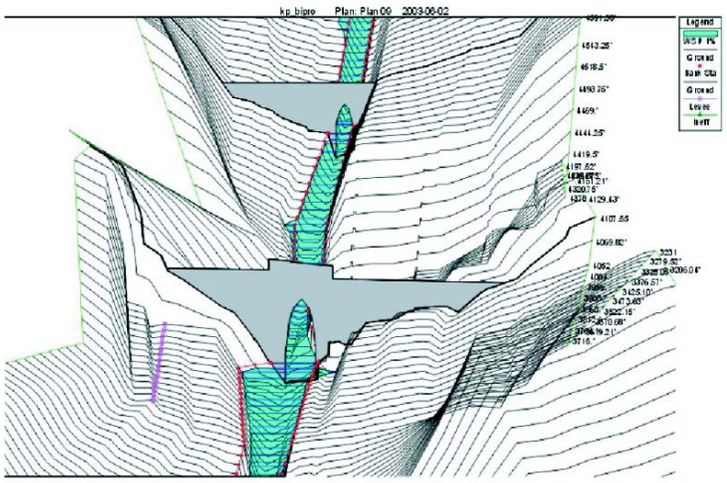
- geodetic description;
- visualization of the topography of an area; and
- a location-altitude map of a floodplain.

ELABORATION OF AN INUNDATION MODEL

Inundation models of floodplains can be developed on the basis of location-altitude maps of an area by using hydraulic models. Boxes 5.14 and 5.15 give examples of floodplain areas for an upland and lowland river.

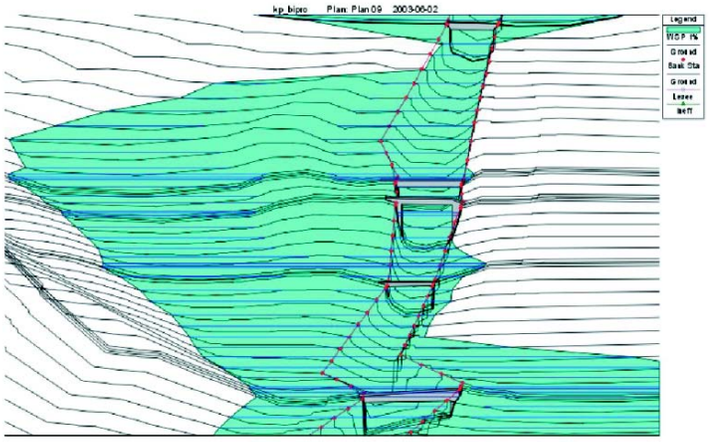
BOX 5.14

Stimulation of the flooded zones on an upland river. The hydrological and ecological capacity of the hydrotechnical construction allows for a 100-year flood



BOX 5.15

Stimulation of the flooded zones on a lowland river; Flood waters flow through grassland areas



MAKE SURE TO CHECK THESE RESOURCES:

Guidelines: chapters 5.E-5.H, 7

www.biol.uni.lodz.pl/demosite/pilica

6.A. BIOASSAYS - A TOOL TO MEASURE ECOSYSTEM QUALITY

Biological monitoring programs are used worldwide to assess river condition. The use of biota to assess river condition has numerous advantages for complex river ecosystem quality assessments. As biotic communities are affected by a multitude of chemical and physical influences, the condition of the biota reflects the overall condition of a whole river ecosystem. This chapter reviews several international biological assessment methods and the potential to use physical assessment methods to complete bioassays.

WHY FOCUS ON RIVER ECOSYSTEM ECOLOGICAL INTEGRITY (EI)?

Over the last decades the main focus of stream and river assessments has been on their chemical/physical water quality. The ability to measure this has been considerably improved in many industrial countries. However, riverine hydrology, morphology and connectivity still continue to deteriorate due to human activities in river basins. Today water directives (e.g., European Union Water Framework Directive, 2000/60/EC) challenges ecologists to provide practical methods for assessing the ecological integrity (EI) of running waters (e.g., FAME project).

Ecological Integrity (EI)
of water ecosystem
(ÕNORM 6232)

Maintenance of all internal and external processes and attributes interacting with the environment insuch a way that the bioticcommunity corresponds to the natural state of type-specific aquatic habitats.

WHY BIOASSAYS?

Despite the availability of many geomorphological/physical assessment methods, there remains an urgent need to develop biologically sound assessments and to link both kinds of methods in a biological perspective.

River bioassays can be based on:

- phytoplankton;
- phytobenthos;
- macrophytes;
- benthic invertebrates; and
- fishes.

Fig. 6.1
Fish-based assessments have the highest power to detect change in riverine ecosystems (photo: Z. Kaczkowski)

BIOASSESSMENT (bioassay)

Uses biota as the endpoint to represent environmental conditions and assess environmental quality.

It has been stressed that as integrators at the highest trophic level in riverine ecosystems, fishes are indicators in river assessments that broaden management objectives towards an ecosystem perspective, e.g., by the Ecohydrology Concept (Zalewski, 2000).

WHAT ORGANISMS CAN BE USED IN BIOASSAYS?

Selection of an indicator group of organisms should consider differences in potential error and accuracy of estimating river status. From this perspective, fish-based assessments are characterized by the highest power to detect change in riverine ecosystems and with the lowest error in this estimation (Box 6.1).

As shown in Box 6.1 indicator variability (δ) decreases along the x-axis in the manner: phytoplankton > zooplankton > macroinvertebrates > macrophytes > fish. Thus, e.g., phytoplankton will have higher α and β error frequencies and, therefore, lower statistical power to detect change than other indicator groups such as fish.

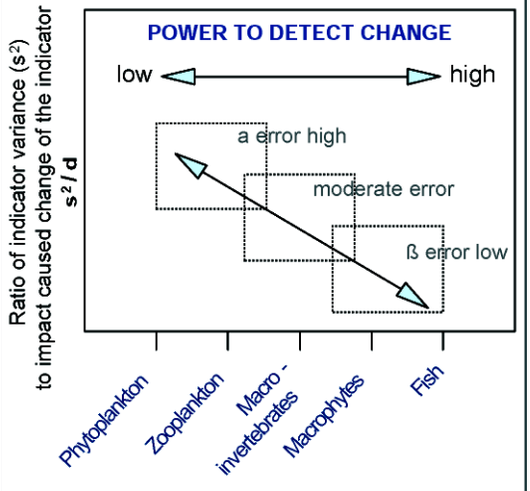
WHY USE VARIOUS INDICATOR GROUPS IN BIOASSESSMENT?

Selection of complementary early- and late-warning indicator groups reduces the probability of not detecting an impact if it occurs.

For instance, phytoplankton has high seasonal va-

BOX 6.1

Bioassessment - errors and accuracy



Conceptual model of hypothetical assessment error estimates for different indicator groups

a error = type I or false positive error:

Selected metric indicate impact when no impact occurs **and than change might not be detected and bioassessment is not proper!**

ß error = type II or false negative error.

Selected metric indicate impact when impact occurs **and than change might be detected and bioassessment is proper!**

(modified from Johnson, 2001)

riability, limiting their use in environmental assessments (high α error) (Box 6.1). Macrophytes have low seasonal variability, but due to slow changes in community structure, can not be used as an early warning indicator. But, when change is detected in macrophyte species composition, then an impact has probably occurred (low β error) - Box 6.1-(Johnson, 2001).

A combination of early-warning indicator groups (phytoplankton, periphyton) together with a late-warning, but statistically more accurate indicator group (macrophytes, fish), results in an optimal assessment of river conditions:

- **if the ecosystem-stressor is nutrient enrichment:**
  consider phytoplankton or periphyton as first choice indicators, as they show a more rapid response to eutrophication than macro invertebrate, macrophyte or fish communities.
- if the ecosystem-stressor is temperature:
  consider fish or macroinvertebrates as first choice indicators, as they show a more rapid response to changes in water temperature than phytoplankton or periphyton communities.

WHAT METHODOLOGICAL APPROACHES CAN BE USED FOR RIVERINE QUALITY BIOASSESSMENT?

The following methodological approaches to bioassessment are currently applied (Johnson, 2001; Faush et al, 1990):

- **single metric approach**: estimates richness, density of individuals, and similarity, diversity of communities (see chapter 6.B.);
- **multimetric approach:** aggregates several metrics as in, e.g., Index of Biotic Integrity (IBI index) for macroinvertebrates or for fish (see chapter 6.B.); and
- **multivariate approach**: measures the mathematical relationships among samples (e.g., similarity in structure of two communities) for 2 or more variables (e.g., qualitative presence-absence of species, or quantitative abundance or biomass of species) are selected. For example, Jaccard similarity coefficient, cluster analysis, discriminant analysis, ordination techniques (PCA, CA, CCA).

Choosing the method, or a combination of methods, should consider method advantages and, especially, disadvantages (Faush et al., 1990):

- multimetric
  - conceptually simple;
  - easy to compare to reference values;
  - More ecologically sound;
  - Dependent on sample size and ecoregion;
  - Easy to understand and interpret and apply by water managers.
- Multivariate
  - Conceptually complex ;
  - Higher precision than multimetric approach; and
  - Difficult to understand and interpret and to apply by water managers;

TABLE 6.1
Bioassessment – examples of methods

ASSESSMENT METHOD	METHOD DESCRIPTION AND FOCUS AND CRITERIA	WHAT IS ASSESSED?
BIOLOGICAL ASSESSMENT APPROACH		
RIVPACS (River Invertebrate Prediction and Classification System) *Evaluated in United Kingdom by Wright et al., (1993)*	▪ uses macroinvertebrate information as the basis upon which to assess the ecological condition of river sites; ▪ the comparison between observed and expected fauna is used to assess the biological guality of sites;	Condition assessment
AUSRIVAS (The Australian River Assessment System) *Evaluated in Australia by Simpson & Norris, (2000)*	▪ uses macroinvertebrate information as the basis upon which to assess the ecological condition of river sites; ▪ macroinvertebrates are collected from reference sites, which are defined as sites representing least impaired conditions.	Condition assessment
IBI (Index of Biotic Integrity) *Evaluated in USA by Karr, (1981)*	▪ multimetric assessment index based on invertebrate or fish assemblages; ▪ employs 12 metrics based on assemblage structure and function (fish or invertebrate assemblages) that give reliable signals of river condition to calculate an index score at a site, which is then compared to the score expected at an unimpaired, comparable site; ▪ uses the "reference condition approach" which involves testing an ecosystem exposed to a potential stress against a reference condition that is unexposed to such a stress.	Condition assessment
HABSCORE (USEPA Rapid Bioassessment Protocols-RPB) *Evaluated in USA by Barbour et al., (1999)*	▪ Rapid Bioassessment Protocols (RBP) that use fish, macroinvertebrates or periphyton to assess stream condition; ▪ this multimetric index represents the biological condition of a site; ▪ physical and chemical data are also measured at each site, and are used to aid the interpretation and calibration of the index, and also to define the reference condition.	Condition assessment
MuLFA (Multi-Level concept for Fish-based, river-type-specific Assessment of ecological integrity) *Evaluated in Austria by Schmutz et al.,. (2000)*	▪ a multi-level concept for fish-based assessment (MuLFA) of the ecological integrity of running waters designed for large-scale monitoring programmes (e.g., European Union Water Framework Directive - WFD); ▪ the principle of the MuLFA is based on assessing the deviation from undisturbed reference conditions; ▪ MuLFA is sensitive to low- and high-dose human alterations, and due to its general character, can be adapted to all river types.	Condition and Ecological value assessment
SERCON (System for Evaluating Rivers for Conservation) *Evaluated in United Kingdom by Boon et al., (1997)*	▪ designed to assess the conservation value of rivers according to criteria of physical diversity, naturalness, representativeness, rarity, species richness and special features; ▪ rating scores are derived for each variable and these scores are subsequently combined to produce indices for each of the conservation criteria; ▪ Field data are collected using the RHS protocols	Ecological value assessment
PBH (Pressure-Habitat-Biota) *Evaluated in New South Wales by Chessman & Nancarrow, (1999)*	▪ has been developed for use in small to medium sized rivers and streams; ▪ measures variables representing the pressures on streams (e.g., physical restructuring, water pollution and introduced species), the habitat of streams (e.g., habitat area, habitat diversity and habitat stability) and the biota within streams (e.g., diatoms, riparian vegetation, macrophytes, macroinvertebrates and fish).	Condition and ecological value assessment

(Dunn, 2000; Phillips et al., 2001; Parson et al., 2002)

METHODS FOCUSED ON BIOLOGICAL ASSESSMENT

The current methods used in bioassessment are described in Table 6.1.

HOW TO LINK ASSESSMENT METHODS IN A BIOLOGICAL PERSPECTIVE?

To improve the quality of river assessment physical and geomorphological, methods in addition to biological methods should be considered (Table 6.2). The common link between assessing river condition from biological and geomorphological/physical perspectives is the use of physical habitat as a template for biological processes and river ecosystem dynamics (Southwood, 1977; Townsend & Hildrew, 1994). Many currently apply bioassessment methods using physical assessment protocols to describe habitat conditions of indicator biota. Recently developed river assessment systems like, e.g., SERCON (Table 6.1), use both biological and physical assessment methods (RHS) to evaluate rivers for conservation.

(See Guidelines: chapters 9.D-9.H)

Table 6.2

Physical and geomorphological assessment – examples of methods

ASSESSMENT METHOD	METHOD DESCRIPTION	LINK WITH BIOTA
PHYSICAL ASSESSMENT APPROACH		
Geomorphic River Styles *Evaluated in Australia by Brierley et al., (1996)*	▪ ability to predict future river character and responses to disturbance, based on geomorphological process theory.	Habitat based links between geomorphology and biota
State of the River Survey *Evaluated in Australia by Anderson, (1993a)*	▪ assessment at many levels - whole catchment, individual sections or individual tributaries, using data components individually or together.	Empirical links between the parameters measured and stream biota (e.g., substratum, riparian vegetation)
RHS (River Habitat Survey) *Evaluated in United Kingdom by Raven et al., (1997)*	▪ assessment of habitat quality of rivers based on their physical structure; ▪ uses a database of habitat requirements, site/reach classifications and association with flora/fauna with different habitats; ▪ at each randomly selected site, a 500 m length of river is surveyed. At 50 m intervals along this length of river, 10 spot checks are performed. A range of features is recorded at each spot check. Data and photos of each sampling site are also stored electronically in a database; ▪ potential linkage with the RIVPACS and SERCON.	Uses the biotope and functional habitat approach to link physical habitat with the biota: The biotope approach is top down in that the use of habitat units by biota is inferred from a knowledge of physical conditions. The functional habitat approach is bottom up in that each habitat is defined from knowledge of the biota that are found in each habitat (Newson et al., 1998a).
IHAS (The Integrated Habitat Assessment System) *Evaluated in South Africa by McMillan (1998)*	▪ measures components of a stream habitat relevant to macroinvertebrates, such as substratum, vegetation and physical stream condition; ▪ measured components are rated and a score representing a continuum of habitat quality is derived.	Assumes that the habitat units are relevant to macroinvertebrate presence
IFIM (The Instream Flow Incremental Methodology) *Evaluated in USA by Trihey & Stalnaker, (1985); Stalnaker, (1993); Stalnaker et al., (1995) under leadership of U.S.Fish & Wildlife Service*	▪ a collection of computer models and analytical procedures designed to predict changes in fish habitat due to increments of flow change. ▪ IFIM software: Physical Habitat Simulation System (PHABSIM), Legal Institutional Analysis Model, Physical Habitat Assessment Model, SALMOD, Stream Network Temperature Model, System Impact Assessment Model.	Assumes that flow-dependent physical habitat and water temperature determine the carrying capacity of streams for fish

(Dunn, 2000; Phillips et al., 2001; Parson et al., 2002).

6.B. FISH COMMUNITIES - INDICATORS OF RIVERINE DEGRADATION

Fish attributes clearly distinguish fishes from other aquatic organisms and underline their significance as essential indicators to assess the ecological integrity of running waters and to estimate their degradation. This chapter presents historical and recent approaches to correctly use fish as a tool in riverine bioassesment.

Fig. 6.2
Fish communities reflect watershed conditions (photo: Z. Kaczkowski)

WHY FISH-BASED RIVER ASSESSMENTS?

„Fish communities reflect watershed conditions", which means that a fish community is a sensitive condition indicator of both an aquatic ecosystem and its surrounding watershed. Because of this, fish communities can be used in biological monitoring to assess environmental degradation (Karr 1987).

Several attributes of fishes underline their essential role as indicators of the ecological integrity (EI) of running waters (after Schmutz et al. 2000a):

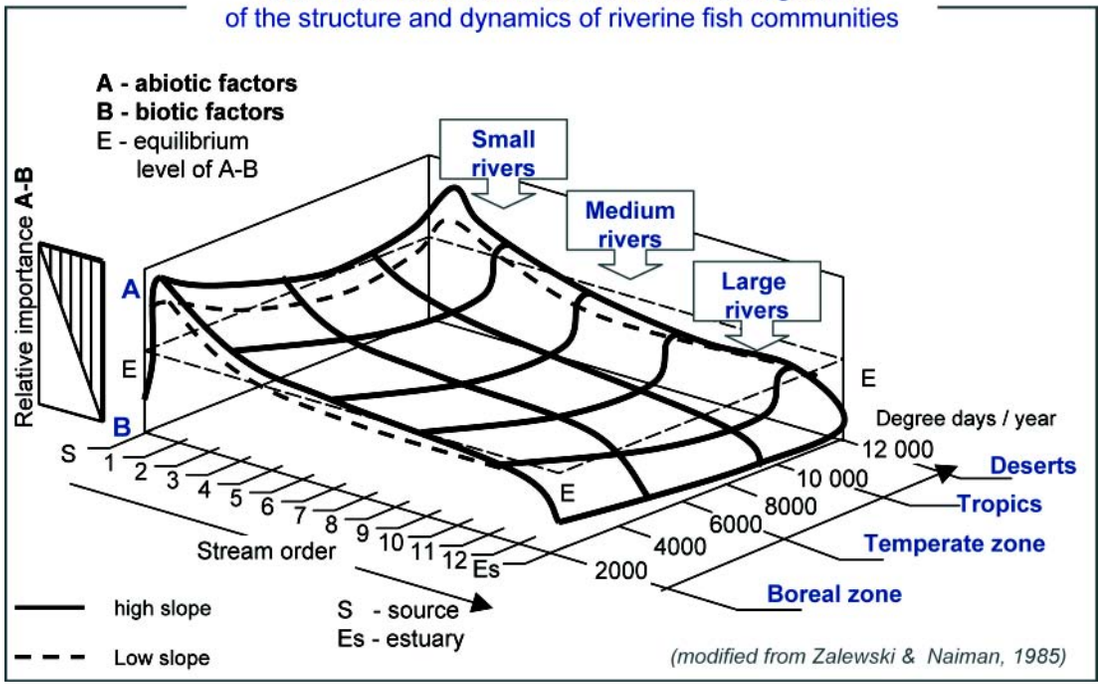
- presence in almost all water bodies;
- well known taxonomy;
- well known life history;
- well known ecological requirements;
- available historical information;
- high habitat preferences make them indicative for habitat quality;
- migratory behavior makes them indicative of river continuum/river connectivity conditions;
- as top predators, subsume trophic condi tions across a food chain;
- as members of a specific trophic guild, provide detailed information on respective trophic levels;
- longevity makes them indicative for long time periods;
- fishery and sport fishing has a long tradition in which fishes have been used as indicators for water quality; and
- economic and aesthetic value helpful in riverhabitat protection and conservation planning.

WHAT ARE FISH-BASED ASSESSMENT CONCEPTS?

The framework on how to use fish communities to describe levels of river degradation is well documented in the multi-level concept for fish-based, river-type-specific assessment of ecological integrity (MuLFA) (Schmutz et al., 2000a). The concept of this assessment method is based on the hierarchical organization of biota (Odum, 1971) and the linkages of the various organizational levels to temporal/spatial scales (Frissell et al., 1986; see: chapter 11.A). According to that theoretical principle, higher levels (fauna or river basin) are more persistent compared to lower levels (individual or microhabitat) and, thereby, less sensitive to degradation than smaller ones. Thus, only a set of assessment criteria selected from different hierarchical levels can guarantee that various human alterations can be detected.

Why taking into account the fish-based river assessment, It should be also considered that , riverine fish community is regulated by a continuum of abiotic-biotic factors, which pressure changes along river continuum and strongly depends on geographical area of the world (Box 6.9). The model described by Zalewski & Naiman (1985) considers a hydrology, slope and climate as major abiotic, and river productivity, predation and competition as major biotic factors. The general assumption of the model is that abiotic factors are of the main importance in all world river types, however while they become stable and predictable the biotic factors start to manifest themselves. Thus, with increase in river spatial hetero-

BOX 6.2
Model of abiotic-biotic factor continuum as regulators of the structure and dynamics of riverine fish communities



geneity, habitat stability and temperature of water, what typically occurs with increasing river size (stream order) the gradually decrease of the influence of abiotic factors, toward biotic control, on fish community should be expected.

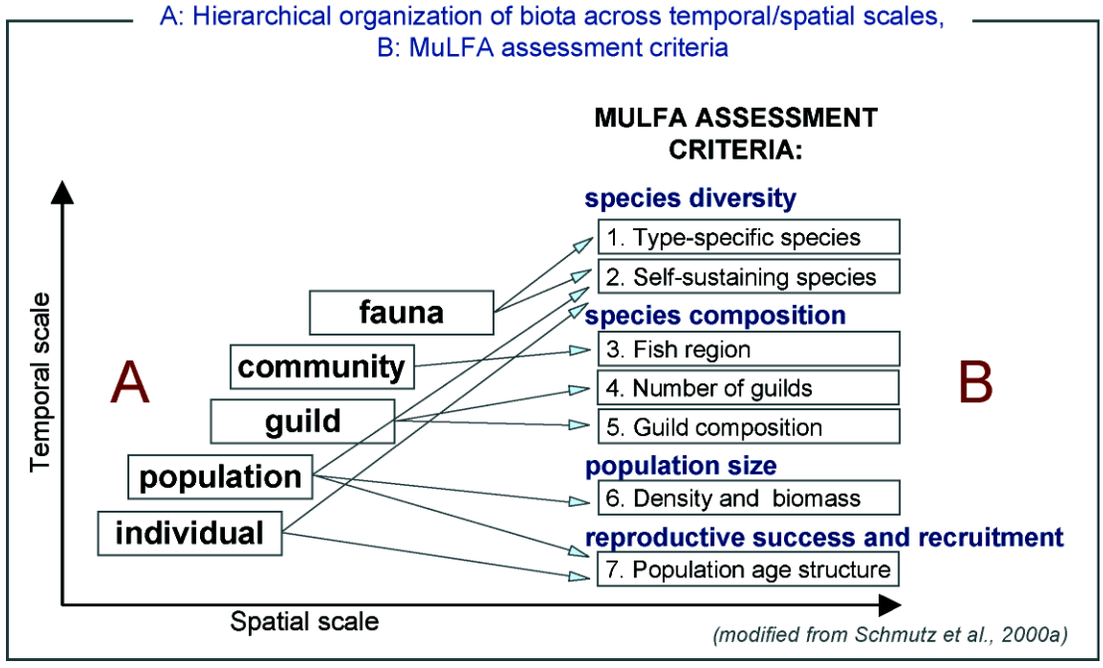
Therefore, to assess the EI of running waters using fish three approaches should be considered: diversity, community and population ones. The MuLFA concept distinguishes seven river assessment criteria (Schmutz et al., 2000a) - Box 6.3, 6.4:

The final assessment procedure is done by comparing an assessment reach with a reference condition reach using a 5-tiered normative scheme (Table 6.3).
MuLFA concept is designed for large-scale monitoring programmes such as required by the e.g. Water Framework Directive of European Community (WFD, (2000/60/EC). And the conceptual approach presented in this chapter is realizing and developing in the research project FAME, supported by the European Commission (Development, Evaluation and Implementation of a Standardised Fish-based Assessment Method for the Ecological Status of European Rivers. A contribution to WFD.)
The MuLFA index is sensitive to low- and high-dose human alterations and can be applied to all river types.

BOX 6.3
Three approaches to assess the EI of running waters

1. Type-specific species 2. Self-sustaining species 3. Fish region	**DIVERSITY APPROACH**
4. Number of guilds 5. Guild composition	**COMMUNITY APPROACH**
6. Density and biomass 7. Population age structure	**POPULATION APPROACH**

BOX 6.4
MuLFA – Multi Level Fish-based Assessment Concept:
A: Hierarchical organization of biota across temporal/spatial scales,
B: MuLFA assessment criteria



(modified from Schmutz et al., 2000a)

TABLE 6.3
MuLFA Index:
5-tiered normative scheme for river integrity assessment

	ECOLOGICAL INTEGRITY LEVELS				
	1	2	3	4	5
CRITERIA	**High** totally or nearly totally undisturbed reference conditions	**Good** slight deviations from undisturbed conditions	**Fair** substantial deviations from undisturbed conditions	**Poor** strong deviation from undisturbed conditions	**Bad** extreme deviations from undisturbed conditions
1. Type-specific species	none or nearly none missing	some species missing	several species missing	many species missing	most species missing
2. Self-sustaining species	none or some missing	several species missing	many species missing	most species missing	nearly all species missing
3. Fish region	no shift	no shift	shift	shift	shift
4. Number of guilds	no guild missing	no guild missing	single guilds missing	many guilds missing	most guilds missing
5. Guild composition	no alteration	slight alteration	substantial alteration	complete alteration	complete alteration
6. Biomass and density	no or nearly no changes	slight changes	substantial changes	heavy changes	extremely changed
7. Population age structure	no or nearly no changes	slight changes	substantial changes	heavy changes	extremely changed

modified from Schmutz et al., 2000a

HOW DOES RIVER DEGRADATION AFFECT FISH SPECIES DIVERSITY?

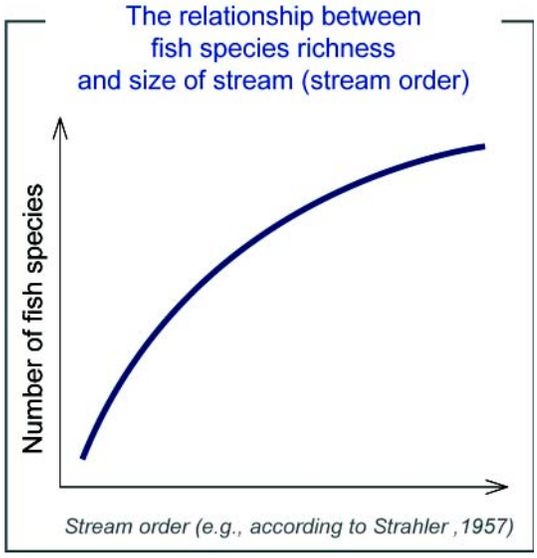
Type - specific species (RTS-species)

RTS - (river type-specific species) criterion reflects the fish fauna naturally occurring in a specific type of a river, excluding species not native in a given area (e.g., country, ecoregion) and not autochthonous for that river.

Generally, the number of native fish species increases with stream order for each type of river (Box 6.5).

BOX 6.5

The relationship between fish species richness and size of stream (stream order)



The main result of river degradation is the reduction of the number of fish species (fish species richness) and decrease in fish community diversity. Fish diversity can be easily estimated by the species diversity index (Shannon & Weaver, 1949). RTS-species criterion is important in situations

$$H` = \sum_{i=1}^{s} n_i/n \log_e n_i/n$$

where fish community diversity is still high, but native species are replaced by non-native species (e.g., introduced), thus indicating river degradation.

Self-sustaining species (SSP-species).

SSP- species criterion reflects the type-specific fauna (RTS-species) composed of species meeting the following minimum criteria: the species are self-reproducing, thus juvenile fishes occur, and maintain, at least a minimum population size.

Minimum population size - at least 50, or better 500, individuals able to reproduce in order to guarantee sufficient genetic variability (50/ 500-rule -Franklin 1980).

HOW DOES RIVER DEGRADATION AFFECT FISH SPECIES COMPOSITION?

Fish regions

A riverine fish fauna can be described as a predictable sequence of distinct communities along a river course.

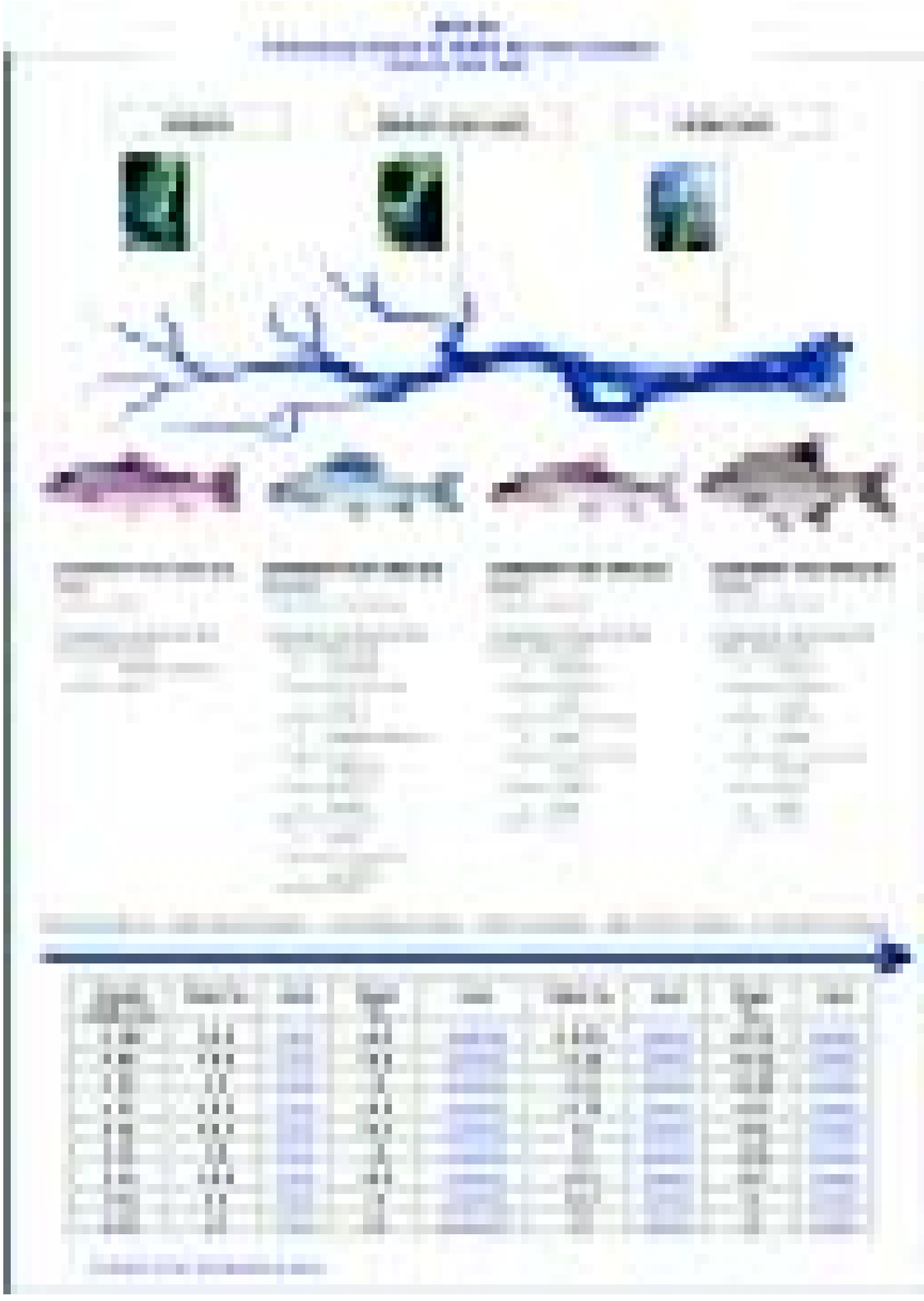
According to two concepts: the fish zonation concept (Thienemann, 1925; Huet, 1949) and the biocoenotic region concept (Illies & Botosaneanu, 1963), fish regions can be classified and named after the dominating key-species, which are associated with other specific fish species of that region:

1. Epirhithral - upper trout region.
2. Metarhithral - lower trout region.
3. Hyporhithral - grayling region.
4. Epipotamal - barbel region.
5. Metapotamal - bream region.
6. Hypopotamal - brackish water region.

Using this classification, the **Fish Region Index (FRI)** can be calculated (Schmutz et al., 2000b). The FRI index estimates the probability of occurrence of key-fish species in a given river region (Box 6.7, Box 6.8).

$$FRI=(3xp3+4xp4+5xp5+6xp6+7xp7)/100$$

River degradation often results in a shift of fish regions to upper or lower regions. Thus, river channelization may cause a «rhithralization effect» in a fish community, or a shift to rhithral zone species. From another site, river impoundments may lead to a «potamalization effect», meaning a shift to potamal zone species (Jungwirth et al., 1995).



BOX 6.7
Fish Region-Index (FRI) - an example

	p3 – epirhitral	p4 – metarhitral	p5 – hyporhitral	p6 – epipotamal	p7 – metapotamal	FRI - index	FRI - index variance
Brown trout	40%	40%	20%	-	-	**3.8**	0.62
Grayling	-	20%	60%	20%	-	**5.0**	0.44
Danube Salmon	-	-	30%	70%	-	**5.7**	0.23
Pikeperch	-	-	-	30%	70%	**6.7**	0.23

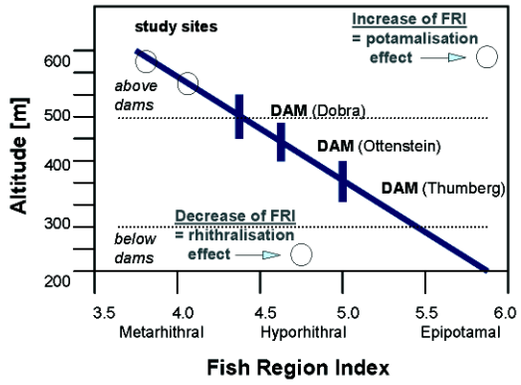
(modified from Schmutz et al., 2000b)

Fish guild number and composition

Guild, in the ecological sense, is „a group of species that exploit the same class of environ mental resources in a similar way" (Root, 1967).

Species are grouped in guilds based on some degree of overlap in their niches regardless of taxonomic relationships. Thus, the guild approach simplifies methodology of fish-based assessment of riverine ecological integrity. And the loss of a fish guild is a much more significant signal of river degradation than the loss of a single species.

BOX 6.8
Fish Region-Index (FRI) - an example



(modified from Kamp, in Schmutz, 2001)

Guild classifications:

1. trophic (Table 6.4),
2. reproductive (Table 6.5),
3. habitat (Table 6.6),
4. residency/migration (Table 6.7),
5. tolerance (Table 6.8),
6. longevity and maturation (Table 6.9).

Guild composition is a commonly used criterion in bioassessment. For example, the Index of Biotic Integrity (Karr, 1981) is constructed upon fish trophic guild composition (Table 6.10 - 6.12).

TABLE 6.4
Fish Trophic Guilds for North American freshwater fishes

TROPHIC CLASS	TROPHIC SUBCLASS	TROPHIC MODE
Herbivores	Particulate feeder	Grazer Browser
Detritivores	Filter feeder	Suction feeder Filterer
	Particulate feeder	Biters Scoopers
Planktivores	Filter feeders	Mechanical sieve Mucus entrapment Ram filtration Pump filtration
	Particulate feeders	Gulping Size-selective pickers
Invertivores	Benthic predators	Grazers Crushers Hunters of mobile benthos Lie-in-wait predators
	Drift predators	Tearers Diggers Surface feeders Water column feeders
Carnivores	Whole body	Stalking Chasing Ambush Protective resemblance
	Parasites	Blood suckers

(modified from Goldstein & Simon, 1999)

TABLE 6.5
Fish Reproductive Guilds
based on spawning habits

I. NON GUARDERS
A. Open substrate spawners
1. Pelagic spawners
2. Benthic spawners
a. Spawners on coarse bottoms
i. Spawners on coarse bottoms with pelagic larvae
ii. Spawners on coarse bottoms without pelagic larvae
b. Spawners on plants
i. Obligate spawners on plants
ii. Non-obligatory spawners on plants
3. Terrestial spawners
B. Brood hiders
1. Benthic spawners
2. Crevice spawners
3. Spawners on invertebrates
4. Beach spawners
II. GUARDERS
A. Substratum choosers
1. Rock tenders
2. Plant tenders
3. Terrestial tenders
4. Pelagic tenders
B. Nest spawners
1. Rock and gravel nesters
2. Sand nesters
3. Plant material nesters
a. Gluemakers
b. Non-gluemakers
4. Froth nesters
5. Hole nesters
6. Miscellaneous-materials nesters
7. Anemone nesters
III. BEARERS
A. External bearers
1. Transfer brooders
2. Auxillary brooders
3. Mouth brooders
4. Gill-chamber brooders
5. Pouch brooders
B. Internal bearers
1. Facultative internal bearers
2. Obligate internal bearers
3. Live bearers

(modifited from Balon, 1975; 1981 a,b)

For the European fish communities the original American IBI should be modified (Table 6.11).

The main effects of river degradation on fish communities in the context of the IBI index are summarized in Table 6.13.

TABLE 6.6
Fish Habitat Guilds

RHEOPHILIC	prefer to live, feed and reproduce in a habitat with high flow conditions, and clear water (e.g., trout)
EURYTOPIC	exhibit a wide tolerance of flow conditions, but generally not considered to be rheophilic (e.g., roach)
LIMNOPHILIC	prefer to live, feed and reproduce in a habitat with slow flowing to stagnant conditions (e.g., the key floodplain species)

(modified from Schiemer and Spindler,1989)

TABLE 6.8
Fish Tolerance Guilds

tolerance capacity of fish species to pollution and environmental degradation, depends on their genetic and physiological constrains	**TOLERANT** **INTERMEDIATE** **INTOLERANT**

TABLE 6.9
Fish Longevity Guilds

live <5 years	**SHORT-LIVED**
live 5 – 15 years	**INTERMEDIATE**
live >15years	**LONG-LIVED**

HOW RIVER DEGRADATION MAY AFFECT FISH POPULATION SIZE

Density and biomass

Fish population size (density and biomass) can reflect river degradation before these impacts start to limit the existence of fish species.

Human alterations can most often be detected as a decrease of population size. But an increase can also be observed (e.g., caused by eutrophication).

The population size of a species should be characterized by quantitative measures - density and biomass per area or river length.

TABLE 6.7
Fish Migration Guilds

1. POTADROMY	occurring entirely within the inland waters of a river system		
2. DIADROMY	occurring across a transition zone between fresh and marine waters	**divided into three sub-categories:**	**ANADROMY** (running up rivers) refers to fishes that live as older juveniles and sub-adults in the sea but at maturity migrate up rivers to spawn, e.g., *Atlantic salmon*
			CATADROMY (running down rivers) refers to fishes that have lived all their early life in fresh water – feeding and growing – but at maturity migrate down rivers to spawn in the sea, e.g., *eel*
			AMPHIDROMY (running between rivers and the ocean) refers to fishes that spend appreciable parts of their life in both fresh and sea waters, feeding and growing in both, and whose migrations seem to have no direct relationship to reproduction

TABLE 6.10
Matrix of the original Index of Biotic Integrity (IBI)

CATEGORY	METRIC	SCORING CRITERIA		
		5	3	1
Species richness and composition	1. Total number of fish species	expectation for metrics 1-5 vary with stream size and region		
	2. Number and identity of darter species			
	3. Number and identity of sucker species			
	4. Number and identity of sunfish species			
	5. Number and identity of intolerant species			
Trophic composition	6. Proportion of individuals that are green sunfish	<5%	5-20%	>20%
	7. Proportion of individuals that are omnivores	<20 %	20-45%	>45%
	8. Proportion of individuals that are insectivorous cyprinids	>45%	20-45%	<20%
	9. Proportion of individuals that are piscivores (top carnivores)	>5%	1-5%	<1%
Fish abundance and condition	10. Number of individuals in sample	expectation for metric 10 vary with stream size and sampling methods		
	11. Proportion of individuals that are hybrids	0%	>0-1%	>1%
	12. Proportion of individuals with externally evident disease, parasites, or other anomalies	<2%	2-5%	>5%

(modified from Karr 1981)

TABLE 6.11
Matrix of the original and modified version of the Index of Biotic Integrity (IBI)

Original Fish IBI (USA)	**General Fish IBI**
1. Number of fish species	1. Number of native fish species
2. Number of darter species	2. Number of riffle-benthic insectivores
3. Number of sunfish species	3. Number of water column insectivores
4. Number of sucker species	4. Number of pool-benthic insectivores
5. Number of intolerant species	5. Number of intolerant species
6. Relative abundance of green sunfish	6. Relative abundance of individuals of tolerant species
7. Relative abundance of omnivores	7. Relative abundance of omnivores
8. Relative abundance of insectivorous cyprinids	8. Relative abundance of insectivorous species (specialized insectivores)
9. Relative abundance of top carnivores	9. Relative abundance of top carnivores
10. Number of individuals	*10. Not a reliable metric*
11. Relative abundance of hybrids	*11. Not often used successfully*
12. Relative abundance of diseased individuals	12. Relative abundance of diseased individuals

(modified from Karr, 1981)

TABLE 6.13
How river fish communities may change with environmental degradation (in context of the IBI index)

1.	DECLINE of the number of all native species and those of specific taxa or habitat guilds
2.	DECLINE of the number of intolerant species
3.	INCREASE of the proportion of tolerant species
4.	DECLINE of the proportion of trophic specialists (top carnivores, insectivores)
5.	INCREASE of the proportion of trophic generalists (omnivores)
6.	DECLINE of fish abundance
7.	DECLINE of the proportion of reproductive guilds requiring silt-free spawning substrate
8.	INCREASE of the possibility of hybridization
9.	INCREASE of disease, parasites, and morphological anomalies
10.	INCREASE of the proportion of introduced species

(modified from Fausch et al., 1990)

TABLE 6.12
Integrity class of the original Index of Biotic Integrity (IBI)

TOTAL IBI SCORE	INTEGRITY CLASS
58 - 60	Excellent
48 - 52	Good
40 - 44	Fair
28 - 34	Poor
12 - 22	Very poor
	No fish

(modified from Karr 1981)

HOW CAN RIVER DEGRADATION AFFECT FISH REPRODUCTIVE SUCCESS AND RECRUITMENT?

Population age structure

Larval and juvenile life stages are often more sensitive than adults to riverine degradation. Thus, reproductive success and recruitment is essential information in river assessments.

The easiest way to assess reproduction might be done by analyzing length-frequency-plots of the population age structure.

HOW TO MONITOR A RIVER FOR FISH-BASED RIVER ASSESSMENT

The concept of fish-based assessment of river quality requires frequent monitoring of the changes in fish communities due to degradation.

How to sample fish?

Electrofishing is world wide tested a very efficient qualitative and quantitative method of fish capture (Cowx and Lamarque 1990). Is possible to catch fish by alternating current (AC), pure direct current (DC) and pulsed direct current (PDC). The main idea of fishing with electricity is based upon the fact that first an electric current attracts fish to the anode (anodic galvanotaxis) and latter reduces fish motion thus makes them easy to catch by net. According to the recent standards (CEN/TC 230/WG 2/TG 4 N 27), either DC and PDC types of electric current may be used, but AC as too harmful for fish should not be anymore considered.

Electrofishing equipment includes: power generator, power conditioner, cathode and one or more anodes (Box 6.9).

Two sampling methods depending on river width and depth should be used (Box. 6.10):

- electrofishing by wading (in small, wadable rivers, usually with 1 anode);
- electrofishing from the boat (in medium size and large rivers, usually 2-3 anodes).

Factors affecting the efficiency of electrofishing

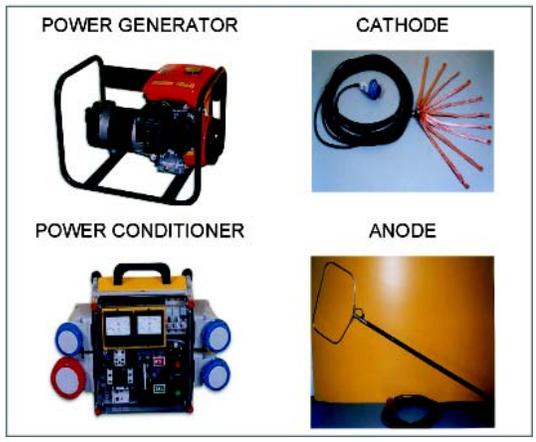
Three groups of factors that affect the efficiency of electrofishing can be selected (modified from Zalewski & Cowx 1990) as shown in the table 6.14.

How is the efficiency of electrofishing estimated?

Results from world rivers of different size and character are shown that the catch-effort electrofishing methods, which are most often employed for estimates of riverine fish density and biomass, are not very precise. Moreover, the multiple electrofishing sampling is both time and manpower-consuming, and what is most important can change both the river habitat and the fish community structure. For above reasons a different fishing procedure can be proposed both to minimalize the

BOX 6.9

Electrofishing equipement



negative effects of electrofishig and to get sufficient results. This approach described by Zalewski (1983) is based on the results collected from different size and character rivers which showed

TABLE 6.14
Groups of factors affecting electrofishing

ENVIRONMENTAL
Abiotic: conductivity (linear relationship), water quality and clarity
Habitat: structure, dimension, bottom substrate, water velocity
Seasonality: temperature, weather
BIOLOGICAL
Community structure: taxocene structure, species diversity and composition
Population structure: density, fish size, age structure, species specific behaviour, physiology, colour and morphology
TECHNICAL
Personel: size of crew, crew experience, motivation and ability
Equipment: design and maintenance
Organisation: site selection, standardization of effort

a curvilinear relationships between the average specimen size and the percentage, number and biomass of fish caught during the first electrofishing (Box 6.11 A, B).

The equation is highly applicable for small and medium size rivers. Above equation was confimed by data from large polish rivers (Penczak & Zalewski, 1973). A section of the 30 m width and 2 m

of average depth river was carefully closed by fake nets and then multiple electrofishing was performed from the boat. Fish capture by fyke nets were considered as analogous to those that can be killed by rotenone treatment which could not be used in this size of river for verification. Two different morphologically river sections were sampled: first a concave bank of meander, and the second the convex bank, more diverse habitat with well develop riparian vegetation in form of overhanging willow branches. The differences in habitat accessibility resulted in about 76% of fish captured during first electrofishing in more uniform river meander and only 51% of fish captured in the convex bank habitat.

In the case of large rivers, the difficulties with application of above method are caused by the variable efficiency of electrofishing in narrow and wide river sections. Thus, two approaches could be proposed: first to divide the wide section into a separate channels with nets, and the second to increase the number of boats and the crew.

BOX 6.10

Sampling methods in rivers – electrofishing

Sampling with electrofishing in shallow rivers

Sampling with electrofishing in deep rivers

(photo: Schmutz, 2001)

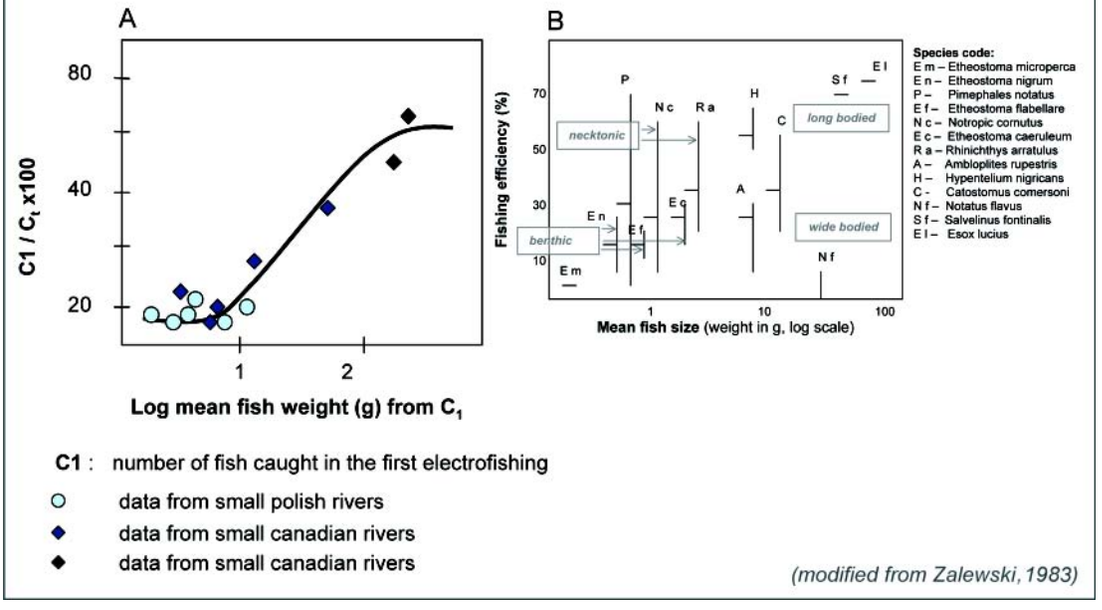
How large sample is required?

The size of the sample should be sufficient to include the home range of the dominant fish species, and encompassing complete sets of the characteristic river form (e.g., pools, riffles, runs) to ensure a good representativeness of the fish community (CEN/TC 230/WG 2/TG 4 N 27)

In order to ensure accurate characterization of a fish community at a given site, the minimum river or stream length to be sampled by electrofishing must be at least 20 times the stream (or river) width (Angermeier & Karr, 1986).

BOX 6.11

Relationship between efficiency of electrofishing and log mean weight of fish (A) and mean fish size (B)



(modified from Zalewski, 1983)

MAKE SURE TO CHECK THESE RESOURCES:

Guidelines: chapters 6, 9

6.C. BACTERIA, FUNGI AND MICROBIAL PROCESSES

Microbial processes are of great importance in the functioning of all water ecosystems.
Results of microbial analyses provide information on rates of decomposition and nutrient cycling in the environment. They give the degree of water contamination, when used as indicators of the sanitary state of watersheds.
Objectives of this chapter are to present specific requirements for microbial sampling and an overview of available methods for microbial analyses from the point of view of their importance for water quality and self-purification.

Fig. 6.3
Bacterial plates
(photo: A. Trojanowska)

WHAT IS THEIR ROLE IN THE ENVIRONMENT?

Bacteria...

Bacteria are the most common and ubiquitous single cell organisms with a great environmental importance. In terms of their metabolism, two groups of bacteria can be specified:

- **Autotrophic** - organisms that obtain energy from sunlight or oxidation of chemical compounds. One photosynthetic bacterial group is the cyanobateria that are common in contaminated watersheds and produce dangerous blooms (see Guideline, chapter 8);
- **Heterotrophic** - organisms that use organic matter as a nourishment source after enzymatic transformation and chemical oxidation. This group is responsible for decomposition processes. They are a crucial element in environmental **nutrient regeneration** cycles of both inorganic and organic **compounds**.

Bacteria play crucial roles in carbon, oxygen and nitrogen cycling in biogeochemical processes through **production and decomposition** of organic matter.

Bacteria are commonly used in biotechnology and bioremediation. However, their pathoge nic activities cause human and plant diseases.

Fungi...

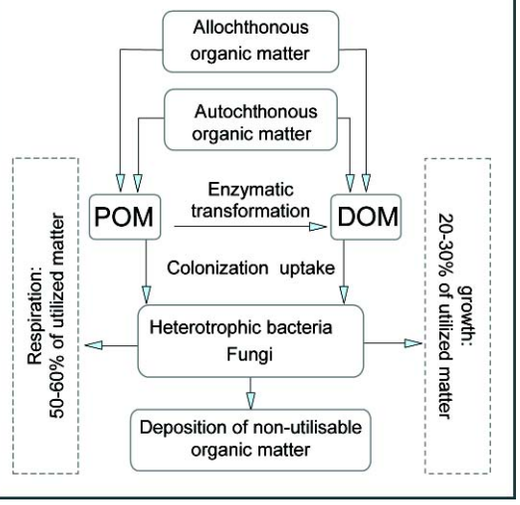
Fungi are ubiquitous and much diversified organisms. Fungi are found in fresh water, marine water, and terrestrial habitats including soil, where they are extremely numerous.

- fungi associated with dead plant matter are important in **cycling of organic matter**, particularly in **degradation** of plant polymers, such as cellulose and lignin, as well as other complex organic molecules.
- fungi are very effective in **bioremediation of heavy metals** and cyclic hydrocarbons (see Guideline, chapter 5). They affect plants beneficially through mycorhizal associations by assisting in nutrient absorption.

COLLABORATION BETWEEN BACTERIA AND FUNGI

Heterotrophic bacteria and fungi act in collaboration creating an efficient system of organic matter decomposition, the so called **„microbial loop"** (Box 6.12).

BOX 6.12
Importance of bacteria and fungi in decomposition of organic matter



This system is responsible for organic matter transformation and mineralization and also liberates inorganic nutrients that are readily available for primary producers. At the same time, microorganisms are utilized by grazers as a food source.
Microbial process rates in fresh waters depend on several abiotic parameters:

- **dissolved oxygen concentration** - decomposition consumes oxygen; a decrease of O_2 concentration below 0,1-0,5 mg L^{-1}, can cause rapid depletion of microbiological process rates.
- **temperature** - at approximately 0°C, the biochemical oxygenation of hard to mineralize organic compounds is nearly stopped. In general, microbial process rates are tempera ture dependent. However, several species are adapted to work effectively in exceptio nally low or high temperature or other extreme conditions.
- **pH** - very sensitive to drastic pH changes.
- the activity of microbial populations depends on the **amount and availability of organic matter**. In some aquatic systems they have been shown to be limited by the availability of inorganic nutrients, especially phosphorus. The total number of microorganisms and their activity (production and respiration) increase together with rising trophic status of ecosystems (Tab. 6.15). In general, the highest values of total bacterial number, as well as their activity, are observed during summer in highly productive ecosystems. However, much higher microbial population densities are observed in sediments (2-3 cm of surface layer) than in water columns.

TABLE 6.15
Population density, production and respiration rates of bacterioplankton in different systems across the trophic gradient

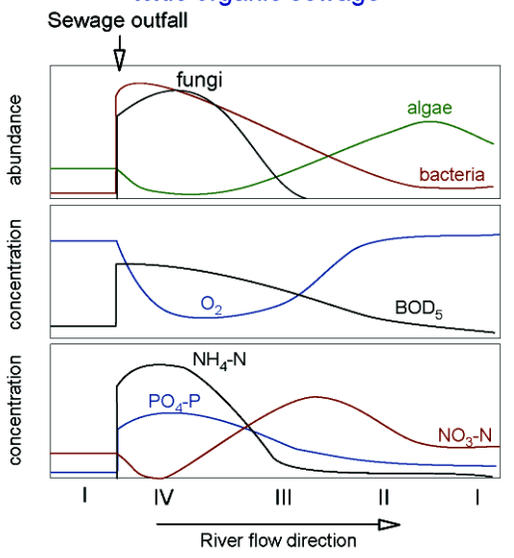
		DENSITY		PRODUCTIVITY	RESPIRATION
		N	B	P	R
hypertrophic	Polluted coastal lagoons	10-40	2000-10000	500-3000	600-50000
eutrophic	Coastal lagoons, marine bays, lakes	5-10	2000-3000	500-1500	600-1700
	Lakes	3-8	600-2000	300-800	380-900
mesotrophic	Internal seas, lagoons, freshwater lakes	1,5-3,0	200-400	100-300	150-400
	Temperate oceanic regions	1,0-2,0	100-300	50-200	100-300
	Antarctic waters (summer-autumn)	1,0	-	-	-
oligotrophic	lakes	0,5-0,8	40-70	15-50	20-60
	Tropical oceanic waters	0,1-0,4	10-30	10-40	15-50
	Intermediate Antarctic waters in the Pacific Ocean	0,1-0,2	10-20	3-5	4-6

N- total number of bacteria (106 cell ml^{-1}), B – biomass of bacterioplankton (mg m^{-3}) and P – production per day (mg m^{-3}), R – respiration of bacterioplankton µg O_2 L-1 day^{-1}

(Sorokin, 1999)

BOX 6.13

Self-purification processes along a river stream polluted by non-toxic organic sewage



Zones: I oligosaprobic;
II ß-Mesosaprobic,
III a Mesosaprobic;
IV Polysaprobic

(modified from Uhlman, 1975)

Self-purification

Microbiological processes are crucial in terms of self-purification of river systems contaminated by domestic sewage. The **self-purification** process is the combined effect of dilution, sedimentation, absorption and biodegradation, which lead to water quality improvement along a stream.

Three main groups of microorganisms taking part in the self-purification process have been identified:

- **polisaprobes** - occupy highly contaminated zones with intensive decay processes;
- **mesosaprobes** - occupy moderately contaminated zones; and
- **oligosaprobes** - clean water organisms.

Their occurrence follows a gradient of decreasing contamination. This phenomenon is used for describing the degree of contamination and rate of self-purification processes along a river according to the saprobic zone classification (Box 6.13).

- **oligosaprobic (I):** upstream of pollution, normal stream conditions, high DO, low BOD, healthy fish, phytoplankton, benthos, periphyton;
- **polysaprobic (IV)**: strongly polluted zone, high bacterial density, very high community respiration, little or lack of photosynthesis, very high BOD, very low DO; fish, benthos, and phytoplankton absent; accumulation of organic particulates, community dominated by sewage fungi;
- **α-mesosaprobic (III)**: high contamination, organic matter being decomposed, community respiration dropping, phytoplankton and photosynthesis recovering, BOD dropping, DO dropping, may be anoxic at night;
- **ß-mesosaprobic (II)**: mildly polluted zone, phytoplankton and macrophytes present, respiration and photosynthesis about equal, DO high, BOD low, biotic communities returning to normal.

The presence of high levels of organic contamination or toxic substances may weaken the condition of microbial communities, decrease their activity and cause self-purification process to be less effective. Self-purification is efficient if the rate of sewage inflow does not exceed a ratio of 1:50 in the receiving waterbody.

Extended buffering zones rich in macrophytes accelerate self-purification in rivers by increasing sedimentation of suspended matter and accumulation of high nutrient loads in plant biomass, which leads to effective elimination of organic contaminants from water. Such systems enriched with macrophytes, according to the ecohydrology concept, are more resistant for anthropogenic stress in terms of increased ecosystem capacity.

BACTERIA AND SANITARY STATE

Microbiological analyses are mostly applied to water sanitary state assessment, which is often a determinant criterion of water quality status. Such analyses are especially required in systems supplying drinking water and water for domestic uses because of the possibility of contamination with infectious microorganisms in case problems occur with the water treatment and/or distribution systems. Tests for detecting and enumerating indica-

tor organisms, rather then pathogens, are used. The density of the coliform group of bacteria is the principal indicator of water pollution and the safety of water for domestic uses.

METHODS OF ASSESSMENT

How to take samples

Samples for microbiological examination must be collected in bottles washed in distilled water and sterilized. Keep bottles closed until they are filled with sample. The volume of sample should be sufficient to carry out all tests required, preferably not less than 100 ml. Protect the bottles from contamination. Leave ample air space in the bottle (at least 2,5 cm) for adequate sample mixing prior to examination.

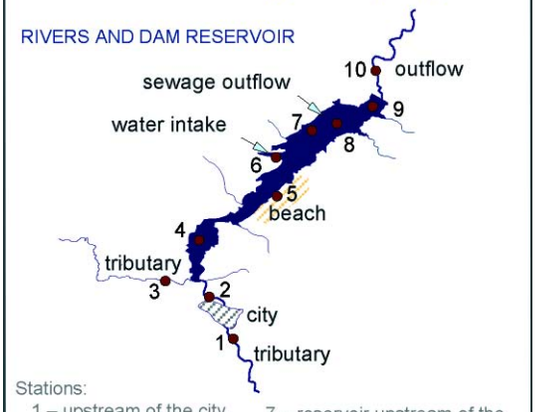
Holding time and storage conditions

Holding time for microbiological samples is only 6 hours (at 4°C) prior to examination or preservation.

TABLE 6.16
Sampling methodology

WATER SYSTEM	SAMPLING METHODOLOGY
Potable water	Put samples directly into the sampling bottle. Open tap fully and let water run for 2-3 minutes. Reduce water flow to permit filling of the bottle without splashing.
Raw water supply	Place the sample directly into the sampling bottle. Obtain samples representative of the water that is the source of supply.
Surface water	Take samples from the surface by putting the bottle, neck downward, below the water surface. Turn bottle until neck points upward and mouth is directed toward the current. If there is no current created, move the bottle horizontally forward but not above the surface. Take care to avoid contact with bank, soil or stream bed. When sampling from a boat, always obtain the sample from the upstream side of a boat.
Bathing beaches	Collect samples from a depth of about 1 m in the swimming area directly by using the sampling bottles.

BOX 6.14
Example of station locations for microbiological sampling



Stations:
1 – upstream of the city,
2 – downstream the city,
2, 3 – tributaries,
4 – reservoir,
5 – beach area,
6 – water intake,
7 – reservoir upstream of the sewage outfall,
8 – downstream of the sewage outfall,
9 – reservoir outflow,
10 – river downstream of the reservoir.

Preservation of samples in 4% final concentration of formaldehyde or ethanol (caution: formaldehyde is toxic - avoid inhalation, ingestion or contact with the skin) is suggested only for samples required for microscopic examination. Samples for examination using culturing methods should be preserved by the addition of a reducing reagent: sodium thiosulfate ($Na_2S_2O_3$) to neutralize residual halogens and to prevent continuation of bactericidal action during sample transportation.

Where to take samples

To monitor stream or lake water quality establish sampling locations at critical sites.

Select bacteriological sampling locations to include a baseline location upstream from the study area, industrial and municipal waste outfalls into the main stream area, tributaries except those with a flow less than 10% of a main stream, intake

points for municipal or industrial purposes, downstream of wastewater outfalls and recreational areas. Notice that sampling downstream of wastewater outfalls should be preliminarily made in horizontal and vertical cross sections to determine the rate of contaminant dispersion (Box 6.14).

Frequency of measurements

Frequency of sampling should be governed by the aim of a study. However, it is recommended to consider a seasonal model of sampling to take into account periods of drastic changes of environmental conditions such as hydrological patterns, temperature, and mixing.

The EPA monitoring requirements for sampling frequency for regulated microbiological contaminants vary depending on the type and size of the system: seasonal for recreational waters and daily for water supply intakes. It is recommended to consider a geometric mean value of at least 5 samples taken over 30 days.

Field and laboratory equipment

All the field and laboratory equipment used for microbiological examination should be washed thoroughly and sterilized.

Sampling apparatus

To collect water samples from depths of a lake or reservoir, ZoBell or Niskin samplers are used. For bottom sediments a standard Van Donsel or any other similar sediment sampler constructed of stainless steel, is applicable.

TABLE 6.17
Selected microbiological methods

CULTURAL METHODS	
Heterotrophic Plate Count (HPC)	Nutrient-poor or nutrient-rich plate or membrane filter method. Provides an approximate number of live heterotrophic bacteria. Number of colonies produced at 20°C in 24 hours is counted and expressed as colony forming units (CFU).
Total Coliform bacteria	Principal indicator of sanitary state, significance and interpretation of test is highly authenticated. Suggested for potable waters. Analysis includes the genera: *Escherichia, Citrobacter, Enterobacter, Klebsiella*. Estimation is made with a multiple-tube lactose fermentation test at 37°C over 24 to 48 hours or on membrane filters incubated on agar plates at 37°C for 24 h.
Faecal Coliform bacteria	Analysis includes the genus *Esherichia coli*. Test applicable to sanitary assessments of streams, raw water pollution and wastewaters. Estimation is made with a multiple-tube enriched lactose fermentation test at 44,5°C over 24 to 72 hours or on membrane filters incubated on agar plates at 44,5°C for 24 hours.
MICROSCOPIC METHODS	
Direct microscopy counting	Involves direct microscopic observations of microorganisms counted in a chamber (such as hemocytometer or Petrof-Hauser) that holds a specific volume of sample. There are special stains used for estimation of fungal and bacterial numbers (acridine orange). The results will be higher compared with cultural methods as a consequence of dead cells and cells not able to grow on culture plates being included.
Epifluorescence microscopy	Standard technique for estimation of total number of bacteria. Sample is treated with fluorochrome (DAPI, fluoresceine isothiocyanete) which is attached to DNA and easily seen as highly fluorescent spots in UV light. Counting is made on black filters after filtrating samples through them. Preserved samples can be stored for up to 6 months before processing.
Advanced microscopic techniques	Scanning confocal microscopy, polarization microscopy or electron microscopy might be used for advanced analyses of bacterial or fungal populations to determine cell structure and chemical composition, for example.

TABLE 6.17 – cont
Selected microbiological methods

MICROBIAL ACTIVITY ASSESSMENT METHODS	
Enzyme assays (selected enzymes)	Enzyme assays used for estimation of specific activity of microbial communities including bacteria, fungi, phytoplankton and actinomycetes: ***Dehydrogenase activity:*** Responsible for oxidation-reduction reactions. Provides information about respiration of microbial communities. Estimation by spectrophotometric method with artificial substrates: Trifenyltetrazolium. ***Phosphatase activity:*** Responsible for hydrolysis of organic compounds containing phosphorus. Provides information about phosphorus mineralization rates. Estimation by spectrophotometric or fluorometric methods with artificial substrates: p-NPP or MUFP (respectively). ***Amninopeptidase activity:*** Responsible for hydrolysis of proteins with liberation of amino acids. Provides information about heterotrophic microbial activity. Estimation using a fluorometric method with artificial substrates: LAKM. ***Protease activity:*** Responsible for hydrolysis of proteins. Measured by determination of residual protein in gelatine medium. ***Cellulase activity:*** Responsible for hydrolysis of plant constituents, releasing monomeric sugar units. Measured by determination of mass loss of cellulose substrates.
Respiration	Indicates rate of organic compound decomposition Measured under anaerobic conditions by CO_2 or CH_4 production or in aerobic conditions by O_2 consumption.
Bacterial production rate	Indicates increase of number or biomass of bacterial population. Measured by: incorporation of radioactive labelled tracers into cellular macromolecules, i.e., thymidine into DNA or leucine into proteins.

Sample bottles

Use glass or plastic bottles than can be sterilized. For some applications pre-sterilized plastic bags might be used.

Laboratory methods

There are several precise and quick methods currently available for:

- estimation of number of live microorganisms - cultural methods;
- direct counting of microorganisms - microscopic methods; and
- microbial activity assessments.

However, their limitations must be understood thoroughly (Table 6.17).

Microbial analyses should be done by a professional microbiologist or by a person who was specifically trained and is periodically supervised by a microbiologist.

INTERPRETATION AND VERIFICATION OF RESULTS

Examination of routine bacteriological samples cannot be regarded as providing complete information concerning water quality. Interpretation of the results should be made in conjunction with chemical and toxicological results obtained at the same time.

STANDARDS:

Existing standards for microbiological testing regard only sanitary state indicators: total coliform, faecal coliform or faecal streptococci bacterial numbers. Remember: In spite of standard methods being used in microbial examination, different limits are used for sanitary state assessments in different countries.

TABLE 6.18
Example of microbiological numerical limits for water of different designations

TYPE OF WATER SYSTEM	LIMIT
Fresh water	126 per 100ml
Salt water	35 per 100ml
Recreational freshwater beach areas	235 per100 ml

Expressed as number of E. coli colony forming units per 100 ml (Faecal Coliform Bacteria).

Water Quality Standards Handbook, EPA-823-B-94-005a, August 1994, Second Edition (Maryland, USA)

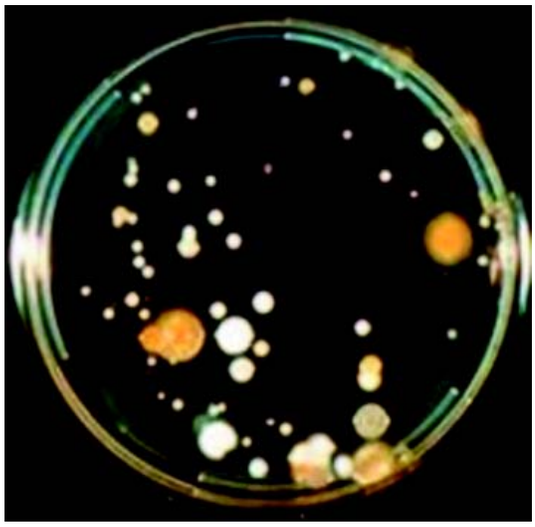


Fig. 6.4
Bacterial and fungal colonies growing on a Petri dish containing nutrient rich medium (photo: Department of Applied Ecology)

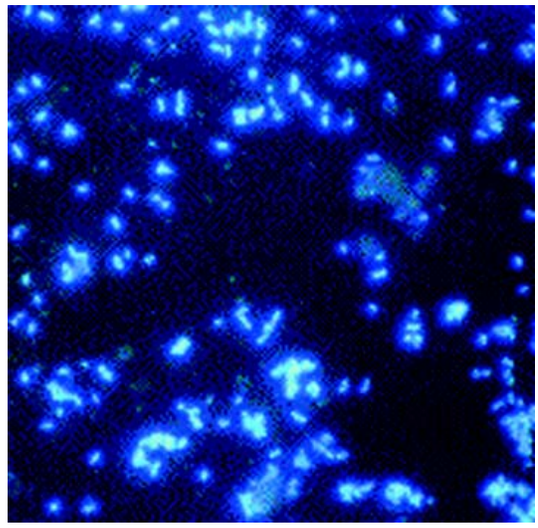


Fig. 6.5
DAPI stained bacterial sample, preparated or counting using a fluorescence microscope (photo: Department of Applied Ecology)

MAKE SURE TO CHECK THESE RESOURCES:

Guidelines: chapters 5, 7.E, 7.G

7.A. WHAT HAPPENS TO PHOSPHORUS IN A WATER BODY: SEDIMENTATION

Sedimentation of matter exported from catchments is one of the serious problems in man-made reservoirs.

Studies of the sediment surface layer provide information on the areas of enhanced sedimentation processes in reservoirs, as well as on the quality and quantity of recently deposited matter. Information obtained from analyses of surface sediments can be used to map the distribution of particular contaminants.

The objective of this chapter is to provide an overview of available methods for sampling and analysis of sediments from the point of view of identifying sedimentation areas and potential risk of internal load.

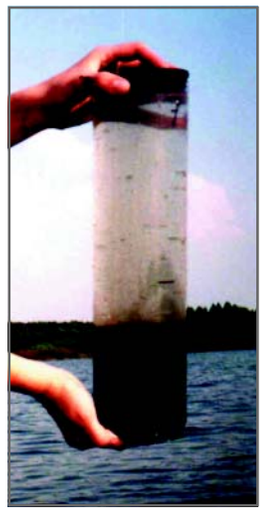


Fig. 7.1
A sediment sample taken by a coring sampler (photo: I. Wagner-Lotkowska)

WHY DO WE MEASURE SEDIMENTATION?

- inflow of solid particles, their sedimentation and deposition causes long-term siltation and decreased capacity of lakes and reservoirs;
- sediments are reservoirs of organic matter, nutrients (mainly phosphorus), as well as dangerous pollutants, such as pesticides, sulphides, ammonium and trace metals, which affect water quality and can cause lethal or sub-lethal effects in benthic communities;
- sediments play an important role in internal loading due to the release of retained nutrients, which can become available to phytoplankton; and
- sediments also provide habitat, feeding and spawning areas for many aquatic organisms.

PROCESSES OF SEDIMENT TRANSPORT AND DEPOSITION

Sediment transport and deposition is a dominant process in reservoirs that significantly influences the ecological state of the ecosystem. Sediment amount, delivery and intensity of its deposition depend on:

- shape, location and land use in the catchment area that determine erosion;
- hydraulic conditions: hydrological pattern, especially storm events, elevated flows and wind action; and
- stream order that determines the amount of allochthonous and autochthonous matter.

The types of matter delivered and deposited with decreasing size of particles are:

- **inorganic particles**: sand, silt, clay; and
- **organic particles**: Coarse Particulate Organic Matter (CPOM), Fine Particulate Organic Matter (FPOM).

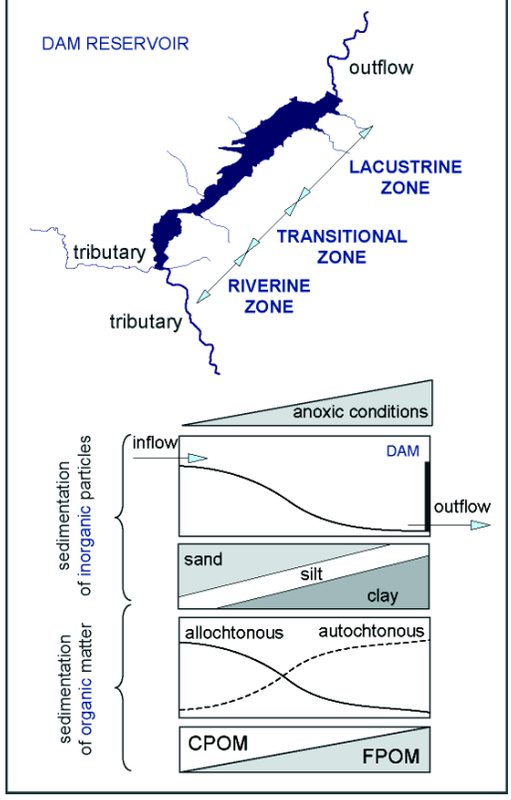
Due to hydrological changes along a reservoir and through a lake, they exhibit longitudinal gradients in suspended sediment concentration, particle size distribution, and in consequence, chemical and biological gradients (Box 7.1).

The rate of deposition is generally higher in a river mouth then in open water areas and higher in lakes dominated by allochthonous, as opposed to autochthonous, matter (highly productive). The grain size of inorganic particles and their rate of deposition decreases longitudinally with the distance from the mouth of the tributary due to a decrease of water velocity.

In terms of the origin of allochthonous organic matter, CPOM dominates in river mouths and riverine zones of reservoirs. However, small particles of FPOM prevail in open waters of the lacustrine zone due to autochthonous algal biomass production.

In terms of sedimentation importance for water quality and capacity of reservoirs, estimation of sedimentation rate and areas of material deposition are highly recommended.

BOX 7.1
Longitudinal gradient of sedimentation in a dam reservoir



EXCHANGE OF NUTRIENTS BETWEEN WATER AND SEDIMENTS

The surface layer of sediments (<5 cm) is characterized by extremely intensive physical, chemical and biological processes of organic matter transformation resulting in:

- durable bonding of nutrients and their retention; and
- liberation of nutrients to interstitial water and transport to the overlying water.

These processes are strongly dependent on water mass stability, temperature and redox potential.

Fluctuating water levels **enhance sediment-water interactions** in reservoirs and results in increased nutrient transport from sediments to the water column. Sudden bottom water movement due to wind mixing or discharge increases, as well as biological **activity of benthic organisms**, cause **resuspension** of deposited particles and facilitate the **return of nutrients** to the overlying water. Similar effects occur at the water-sediment interface in anoxic conditions, which are observed during high temperature periods in nutrient rich ecosystems or in nutrient-poor tropical and subtropical systems. When the oxygen concentration decreases to below 2 mg L^{-1} (redox potential <200mV) phosphorus, iron, magnesium and ammonia are liberated and transported to the overlying water. The above processes of internal loading significantly contribute to increased nutrient availability for algae and cyanobacteria.

METHODS OF ASSESSMENT

How to collect the samples

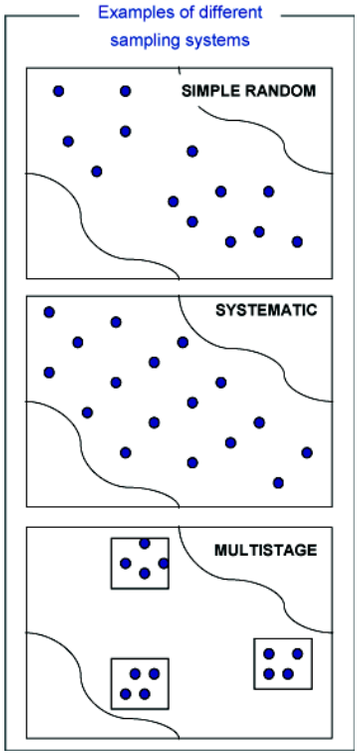
- sample **volume** should be obtained by consulting with a testing laboratory to confirm the amount of sediment required for analysis. If full biological, toxicological and biological testing is required, at least 10 litres of sediment might be required from each station;
- consider taking **integrated** samples from a given station or across similar station types to reduce the number of samples needed;
- additional field observations and measurements are important when sediment sampling:
  - coordinates;
  - oxygen concentration measured at the sediment-water interface; and
  - pH and temperature in water overlying the sediments.
- to minimize measurement error:
  - sample all stations similarly within a study;
  - use standardized procedures;
  - sample during the same time period; and
  - collect and analyse multiple samples at a station.

Where to collect the samples

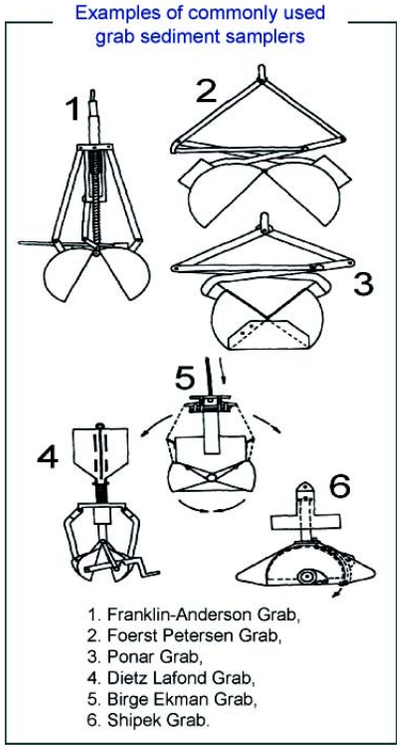
Design a sampling net relevant to the aim of the study. The following types of sampling systems can be distinguished:

- **deterministic system**: based on given information or purposes, usually denser in areas of special interest;
- **stochastic**: based on random sampling;

BOX 7.2
Examples of different sampling systems



BOX 7.3
Examples of commonly used grab sediment samplers



- **regular grid system**: which can be placed randomly or deterministically on a lake (Box 7.2)

Furthermore, the selection of sampling sites should take into consideration the location of critical points affecting sediment quality:

- sediment depositional zones;
- tributaries;
- water intake areas;
- sewage outfalls; and
- location of historical sampling stations.

Frequency of measurements

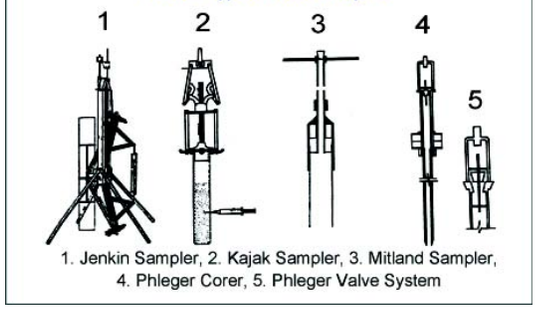
Frequency of sampling should be governed by the study aims. However, it is recommended to consider a seasonal model of sampling with respect to periods of drastic changes of hydrological pattern, including floods, storm events, elevated flows and droughts.

Sediment samplers

A large number of sediment samplers have been designated for specific purposes and for sampling in different environments. Most sediment samplers can be classified as core (does not disturb sediment profile) or grab samplers (Box 7.3, Box 7.4). In most cases special decontamination of sampling equipment is not required; rinsing in water between sampling stations should be enough. However, if at least one of many sampling stations is heavily contaminated, it might be necessary to decontaminate all sampling devices using the following steps:

- washing in soap and water;
- rinsing in distilled water;
- rinsing in acetone or ethanol; and
- rinsing in site water.

BOX 7.4
Various types of core samplers



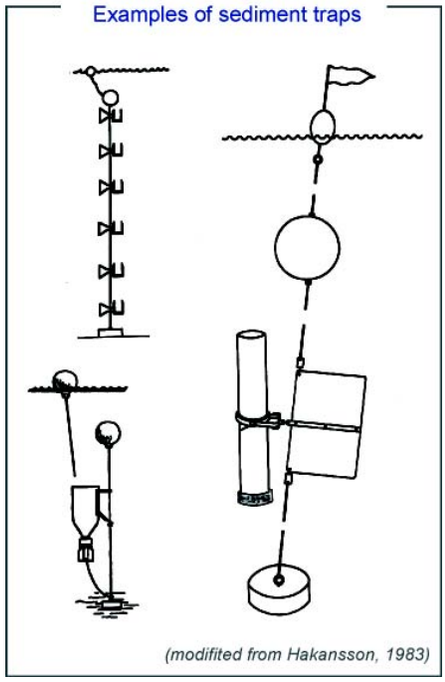
Sediment traps

Sediment traps are used for measuring sedimentation rate. Usually several traps, such as cylinders, bottles or funnels, are submerged to collect sedimenting particles over a certain time period (Box 7.5).

Sample containers

Borosilicate glass, high density polyethylene (HDPE) or polytetrafluoroethylene (PTFE) containers are suitable for most analytical measurements. All containers should be pre-cleaned prior to filing with the sample. Purge containers with inert gas (nitrogen) prior and after filling if anoxic conditions must be maintained. Fill containers completely if the sample will not be frozen.

BOX 7.5
Examples of sediment traps



(modifited from Hakansson, 1983)

Methods

Assessment of the physical-chemical quality of sediments, combined with toxicity testing, is often the most frequently required information. Chemical analyses should be done by qualified chemists in a professional chemical laboratory.

Sample transport and storage

The volume of overlying water should be minimized to reduce potential resuspension. Samples should be secured to avoid sample disturbance. According to general recommendations, collected sediment samples should be stored in containers without a headspace at 4°C in the dark to minimize changes in contaminant bioavailability. However, preservation and storage times for samples designed for various types of analyses are different (Appendix 1).

Examples of field forms for sediment sampling are given in Appendix 2.

Interpretation and verification of results

Different standards and different limits are used in different countries for assessment of sediment quality and sanitary state. The most frequently used parameter for sediment quality characterization are the contents of trace metals and organic cyclic compounds.

As an example, Appendix 3 presents selected parameters and standards according to the USEPA.

TABLE 7.1
General considerations for core and grab samplers usage

USE A CORE SAMPLER IF:	USE A GRAB SAMPLER IF:
- Characterization of contamination in deeper sediments is important - Comparison of recent surficial and historical deeper sediments is intended to be done - Reduced sediment gradient disruption needed - Reduced oxygen exposure needed - Sediment is soft and fine grained	- Large sediment volumes needed - Larger grained sediments are common - Larger surface area of surficial sediment needed

MAKE SURE TO CHECK THESE RESOURCES:

Guidelines: chapters 7, 8.A

7.B. WHAT HAPPENS TO NITROGEN IN A WATER BODY: DENITRIFICATION

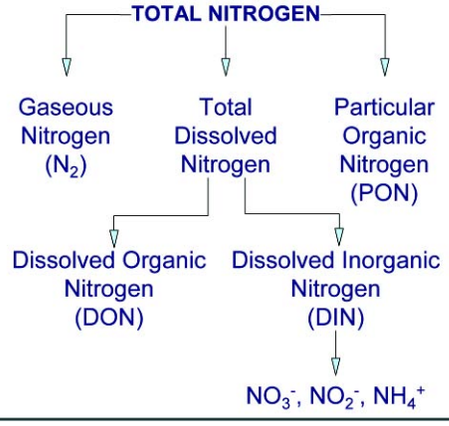
Nitrogen is one of the factors limiting algal growth in rivers and lakes. The principal requirement of living cells for nitrogen is synthesis of aminoacids and proteins. Nitrogen surplus causes eutrophication, but also such environmental problems as acid deposition, global warming and depletion of the ozone layer. Because of the gaseous cycle, methods of nitrogen control are different from those for phosphorus and other nutrients. The objective of this chapter is to present methods of assessment for the rate of N-cycling in fresh water.

THE MAJOR FORMS OF NITROGEN IN FRESHWATER ECOSYSTEMS

Nitrogen is present in fresh water in many forms (Box 7.6, see guidelines: chapter 7.G). However, only the reduced form (NH_2^-) can be built into organic macromolecules, mainly amino- and nucleic acids. After carbon and oxygen, nitrogen is quantitatively the most abundant compound in organisms, constituting 15-20% of their dry weight.

BOX 7.6

What are the forms of nitrogen in fresh waters?



Nitrogen fixation brings N from the atmosphere into the biosphere and denitrification returns N to atmospheric N_2. Due to disturbances of the N-fixation/denitrification balance, the turnover of higher amounts of NO_3^- to N_2O will further destroy the ozone layer to the stratosphere and extend the greenhouse effect.

Fig. 7.2
The process of denitrification occurs intensively in anaerobic environments of oxbows and floodplains (photo: B. Sumorok)

MAN-MADE SOURCES OF NITROGEN

The main sources of nitrogen contamination are:

- transboundary atmospheric pollution (acid rain);
- oil pollution;
- agricultural ground water pollution, resulting mostly from nitrate fertilizer use;
- faecal contamination; and
- domestic and industrial sewage water pollution.

WHAT ARE THE EFFECT OF NITROGEN ENRICHMENT?

The enrichment of soil and surface waters with nitrate may endanger the balance of the natural environment or even restrict environmental resources from use due to the accumulation of toxic nitrite products. The most dangerous effects of nitrogen enrichment include:

- **toxic algal blooms**, appearance of which may restrict use of freshwater resources; and
- **temporal accumulation of nitrate**, the consumption of which in potable water can cause infant methemoglobinemia (blue baby syndrome).

According to WHO recommendations, the maximum allowable concentration of nitrate nitrogen in potable water, should not exceed 10 mg L^{-1} (WHO, 1971).

THE ROLE OF SEDIMENTS IN NITROGEN CYCLING

Bentic metabolism plays an important role in the regulation of nutrient concentrations and, thus, the productivity of ecosystems. In the case of ni-

trogen, depending on the physical and chemical parameters, sediments can be both a source as well as a major sink in the cycle of this element. The regeneration of ammonium in sediments is a major source of nitrogen to the water column, whereas production of dinitrogen gas (denitrification) and burial are major nitrogen sinks. Most of the organic matter reaching the sediments is microbially degraded in the ammonification process. Box 7.7 presents a schematic illustration of the nitrogen cycle in sediments. The name of different N-cycle processes are in *italics*.

WHY DO WE MEASURE DENITRIFICATION?

The denitrification process is responsible for removing nitrogen from wastewater and eutrophicated reservoirs and lakes. The process may be additionally enhanced by regulation of the physical characteristics of the site. Therefore, it can be used for nitrogen removal from rivers, lakes and reservoirs, as well as in transitional land-water zones. It is important to identify areas of intensive denitrification in order to control the process for nitrogen removal (Table 7.2).

TABLE 7.2
Main nitrogen transformations in water

PROCESS	PATHWAY	REGULATED BY	ORGANISMS TAKING PART IN THE PROCESS:
Assimilatory nitrate reduction	$NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$	NH_4^+, organic N	plants, fungi, algae, bacteria
Denitrification	$NO_3^- \rightarrow NO_2^- \rightarrow N_2O \rightarrow N_2$	O_2, organic C	aerobic bacteria also capable of anaerobic growth with NO_3^- or NO_2^-
Dissimilatory nitrate reduction to ammonium (Ammonification)	$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow NH_2OH \rightarrow NH_3$	O_2, organic N	anaerobic and facultatively anaerobic bacteria (for example, *Bacillus* sp., *Aerobacter* sp.,)
Nitrification	$NH_3 + 1{,}5O_2 \leftrightarrow NO_2^- + H^+ + H_2O$ $NO_2^- + 0{,}5O_2 \leftrightarrow NO_3^-$	O_2, inorganic C (CO_2, HCO_3^-)	bacteria (*Nitrosomonas* spp., *Nitrobacter* spp.)
N_2 biological fixation	$N_2 + 3H_2 \rightarrow 2NH_3$ $2CO_2 + H_2O + NH_3 \rightarrow CH_2NH_2COOH$	Enzyme system: *nitrogenase;* under anoxic conditions	Prockaryota: cyanobacteria, bacteria

BOX 7.7
Main nitrogen transformations in water

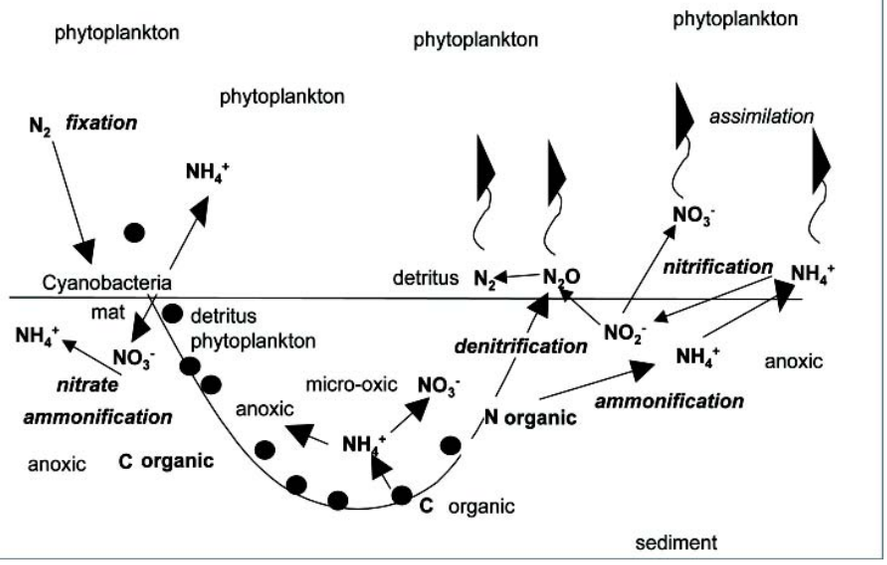


TABLE 7.3

Summary of methods used to measure denitrification activity

METHOD	ANALYSIS	WHERE USED	COMMENTS
Acetylene inhibition – N_2O production	Gas chromatography	Natural samples of all types; microbial cultures	The most popular method because of its sensitivity, capacity for large number of samples, simplicity, low cost, and does not require any addition to the natural NO_3^- pool. Its limitation is that acetylene also inhibits nitrification and thus will reduce the denitrification rates in samples where NO_3^- is very low.
N_2 production	Gas chromatography	Microbial cultures; used for natural samples	Because of large N_2 background in the atmosphere – the sample atmosphere has been exchanged for another gas (e.g., He). The sample must be very active for detection of denitrification over leaks, out-gassing, and relative insensitivity of the thermal conductivity detector.
Consumption of NO_3^- and NO_2^-	Colourimetric methods, nitrate electrode, steam distillation, UV adsorption (direct or after HPLC)	Microbial cultures; aquatic habitats	Not considered definitive for denitrification since NO_3^- and NO_2^- can be reduced to NH_4^+. Can be used together with total NH_4^+ + organic-N (mass balance), in which unrecovered N is equated to denitrification. The method is relatively insensitive and works best when N_{tot} in the sample is very low.
NO_3^- / Cl^- (Br^-) ratio	Colourimetric methods, specific ion electrodes,	Soil	Both anions have the same mobility in natural systems, but only NO_3^- is consumed. The method is rather qualitative, but it has the advantage of requiring only a single measurement and it reflects the process as it occurred naturally.
N_2 /Ar ratio	Gas chromatography (thermal conductivity)	Marine water column	The method is not sensitive, but it directly reflects the process over a large scale in nature. N_2 consumption by fixation can confound this measurment, so it is usually not significant in the deeper water column where this method is used.

TABLE 7.3 – cont.

Summary of methods used to measure denitrification activity

METHOD	ANALYSIS	WHERE USED	COMMENTS
Isotopic Methods **The ^{15}N isotope**	Mass spectrometry magnetic sector quadrupole UV emission		Mass spectrometry is the preferred analytical method. Isotope ratio instruments are designed for high precision measurements, but most require a large amount of N_2. The quadrupole units are coupled to a gas chromatograph and have the ability to separate gases as well as detect much smaller amounts. The UV emission instrument is less expensive and requires only small amount of N_2, but is much less precise.
Isotopic Methods **^{13}N**	β^+ liquid scintillation, proportional counter γ NaI (Tl) detectors		^{13}N is the longest lived radioactive N isotope; It provides the greatest sensitivity of any denitrification method, but it can be used for only short periods, must be used near an accelerator, and requires expensive equipment. It is the only direct method to measure denitrification in the natural atmosphere.
Use of isotopes **Labelled N-gas production** **$^{13}N_2$, $^{13}N_2O$, $^{28}N_2$, $^{29}N_2$, $^{30}N_2$** **Mass balance (loss of enriched ^{15}N or depleted ^{14}N nitrogen)**		Natural sample, culture Natural samples, cultures	Isotopes can be used to improve the sensitivity and specificity of denitrification measurement. A limitation is that the isotope must be uniformly mixed with the natural pool over space and time and in amounts that will not increase the natural nitrate concentration.
Isotope dilution **Using ^{15}N**	Mass spectrometry	Natural samples	Isotope dilution to measure denitrification can be used by observing dilution of ^{15}N-enriched atmosphere or by the use of modelling in conjunction with the measurement of other N-cycle processes.
Microbiological method: occurrence of denitrifying bacteria	Determined by means of the most probable number (MPN) and plate counting (PC).	Sediments, surface waters, soils, wastes	The highest percentage of denitrifiers in relation to the total number of bacteria at selected environmental sites is found at stations where the content of organic carbon was also the highest.

(modified from Tiedje, 1982)

WHERE AND HOW SHOULD DENITRIFICATION BE MEASURED?

Table 7.3 presents summary of methods used to measure denitrification activity.The denitrification rate in natural samples should be measured in summer or early autumn. The measurement stations with the highest denitrification rates in the bottom sediments are located near islands and in bays of reservoirs where beneficial conditions arise from the accumulation of organic matter.

The most useful methods to measure denitrification in the field are:

- **the *in situ* chamber method** - the denitrification rate is calculated from the total N_2 flux out of the sediment measured directly by gas chromatography (Box 7.8); and
- **occurrence of denitrifying bacteria** - determined by means of the most probable number (MPN) and plate counting (PC).

In situ denitrification measurements

This method is the most useful for shallow, nutrient-rich reservoirs. The denitrification rate is measured in summer or early autumn and calculated from the total N_2 flux out of the sediment and calculated as (mol N_2 m^{-2} h^{-1}; Bednarek et al., 2001. Sediment cores are collected, dried and subjected to chemical analysis for organic matter, organic carbon and nitrogen as denitrification reaction substrates. The results are calculated in µg C g^{-1} of dry weight of sediment.

Microbiological analyses

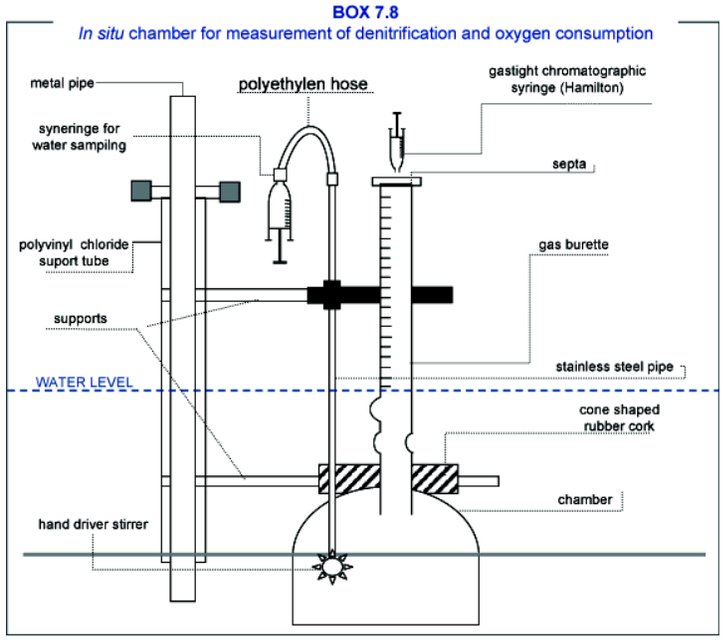
For comparison of denitrification rates using the *in situ* chamber method, sediment samples for bacteriological testing should be collected at the same sampling stations.

Bacterial numbers and isolation of denitrifers. Occurrence of denitrifying bacteria is determined by means of the most probable number (MPN) and plate counting (PC) methods (Gamble et al., 1977). Strains of denitrifying bacteria are isolated from a bacterial colony growing on nutritive agar. About 100 colonies from selected dilutions are replicated and tested for the presence of gaseous nitrogen during nitrite reduction in nutrient agar supplemented with 345 mg $NaNO_2$ L^{-1}.

Identification of denitrifying bacteria. Identification of denitrifying bacteria is performed according to Grama's method, which produces fluorescently pigmented colonies on King's A and B media, from starch hydrolysis in the presence of cytochromium oxidases (Burzynska, 1964). API 20 NE (bioMerieux) is a standardized micro-method combining 8 conventional tests and 12 assimilation tests in prepared kits.

BOX 7.8

In situ chamber for measurement of denitrification and oxygen consumption



7.C. HOW TO ASSESS PHYTOPLANKTON BIOMASS?

Massive phytoplankton growths are one of the effects of eutrophication in lakes and reservoirs. This is because nutrient availability is a major factor limiting phytoplankton growth. Therefore, measurement of phytoplankton biomass is one of the parameters used to assess the trophic level of a water body. It is also a warning indicator about the possible appearance of toxic cyanobacteria.

The objective of this chapter is to outline methods for the quantitative assessment of phytoplankton and eutrophication levels using phytoplankton analysis.

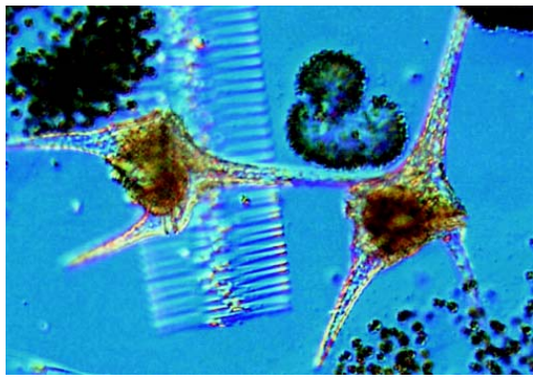


Fig. 7.3
Ceratium hirundinella
(photo: P. Znachor)

WHY SHOULD WE MEASURE PHYTOPLANKTON BIOMASS?

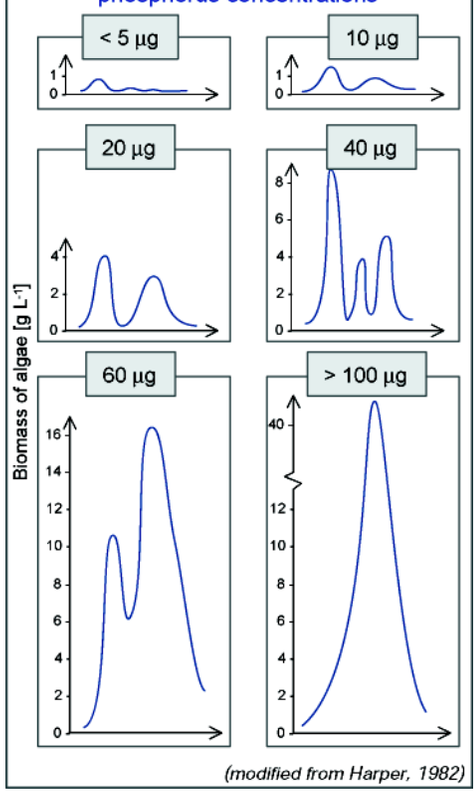
The structure of phytoplankton communities in aquatic ecosystems is dynamic and constantly changing during a growing season, both in species composition and biomass distribution. Many factors are responsible for phytoplankton succession (Box 7.9, Box 7.10):

- **abiotic factors**
  - temperature;
  - irradiance;
  - hydraulic throughput;
  - mixing and stratification dynamics;
  - water retention time;
  - pollutants; and
  - nutrient availability.
- **biotic factors**
  - selective predation by zooplankton;
  - interspecies competition for limiting resources; and
  - parasitic populations.

As a consequence of differences in latitude, climate and stratification patterns, phytoplankton succession may be different between different water bodies, even in one region. In the temperature and polar zones there is a great contrast between summer and winter and in the tropics between the rainy and dry seasons.

The phytoplankton communities in temperate lakes show seasonal variation with a minimum during winter. Maxima are reached during spring and fall mixing and, in many lakes, also during late summer (Box 7.9). In tropical water bodies, high phytoplankton biomass can occur throughout the year.

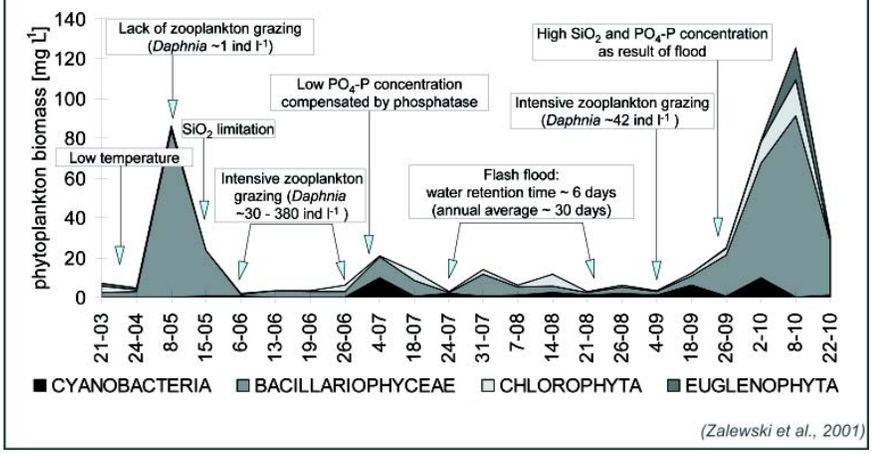
BOX 7.9
Diagrammatic representation of phytoplankton biomass changes in lakes with increasing phosphorus concentrations



Knowledge about the hierarchy of factors that are responsible for phytoplankton succession and species domination in a reservoir is a valuable tool for effective management of these water resources.

BOX 7.10

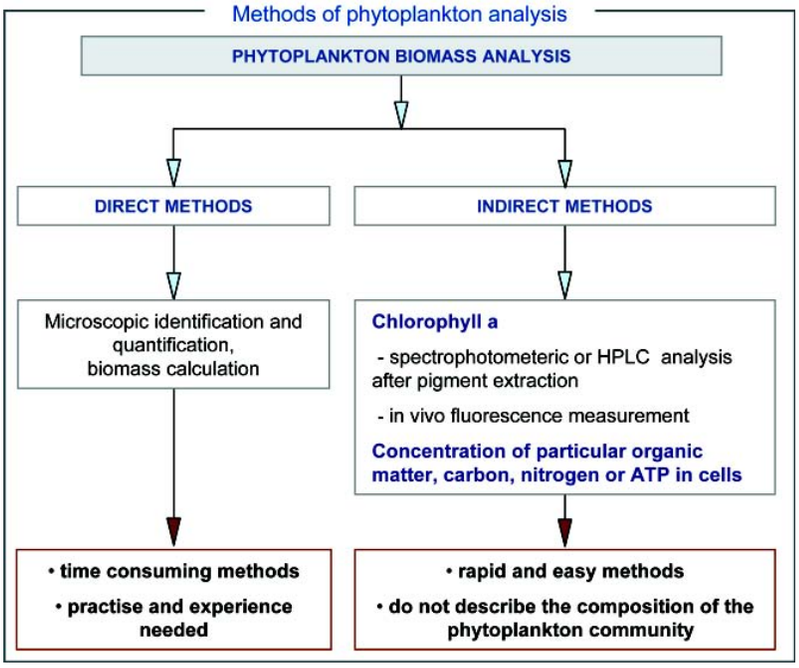
Sequence of abiotic and biotic factors that regulate the occurrence and biomass of phytoplankton in the Sulejow Reservoir, Poland



(Zalewski et al., 2001)

BOX 7.11

Methods of phytoplankton analysis



HOW TO ANALYSE PHYTOPLANKTON

Box 7.11 presents direct and indirect methods of phytoplankton analysis

Number and location of stations

The location of sampling stations may involve several points distributed horizontally over a water body. The number of sampling stations in a lake or reservoir depends on:

- **purpose of sampling**: for a preliminary survey it may be sufficient to collect samples at a single station in the centre of the lake.
- **morphometry of lake**:
  - for lakes of regular shape, the station should be located in the centre of the lake;
  - for lakes of irregular shape with several large bays, these should be sampled separately using at least 1 station; and
  - for man-made reservoirs, samples should also be taken near the inflow and dam wall.

At which depths should we sample?

- in **shallow lakes** (maximum depth < 3 metres) collection of surface samples may be sufficient;
- in **deeper lakes** (maximum depth > 3 metres) it is recommended to take integrated vertical samples. According to standard procedures, samples should be collected from:
  - surface;
  - 1/3 of the depth of a lake;
  - 2/3 of the depth of a lake; and
  - 1 metre above the sediments.
- in **stratified lakes**, samples are taken from:
  - epilimnion (1 metre depth);
  - metalimnion (at the depth of the greatest temperature gradient); and
  - hypolimnion (1 metre above the sediments)

The sample volume should be adapted to the trophy of a lake:

- for eutrophic: 0.5 - 1 litres;
- for mesotrophic: 2 - 5 litres; and
- for oligotrophic: 5 - 10 litres.

How often do you have to sample?

- according to recommendations of many **standard** environmental monitoring programmes, a minimum of 12, or monthly, samples is recommended;
- for **advanced** qualitative or quantitative analyses of phytoplankton, the sampling frequency should be higher - weekly or biweekly during the open water season; and
- during **occurrences of harmful or noxious algae**, sampling should be done at least twice a week.

Field equipment

For phytoplankton investigations a tube sampler is recommended (Fig. 7.4). The sampler should be equipped with a cord with a depth measuring scale and a weight to close the closing devise on top of the sampler. This type of sampler can be adapted for use at all depth intervals.

Plankton nets are not recommended for either qualitative or quantitative analyses of phytoplankton, since a large percentage of important algal species are much smaller than the mesh size of even the finest mesh size. In an addition, fragile species can be broken and pass through nets.

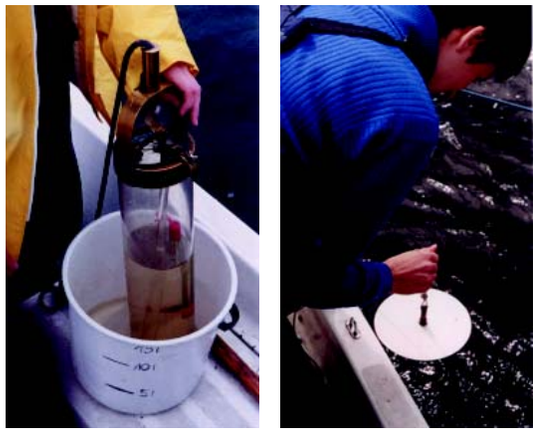


Fig. 7.4
Tube sampler and Secchi disc
(photo: M. Izydorczyk)

MICROSCOPIC ANALYSIS OF SAMPLES

The best tool for quantitative analysis of phytoplankton is a microscope. Counting procedures are similar whether a sedimentation chamber with an inverted microscope (Fig. 7.5) or slides or counting cells with regular microscope, are used.

Cell volumes are calculated for each species from formulae for solid geometric shapes that most closely match the cell shape based on cell dimensions.

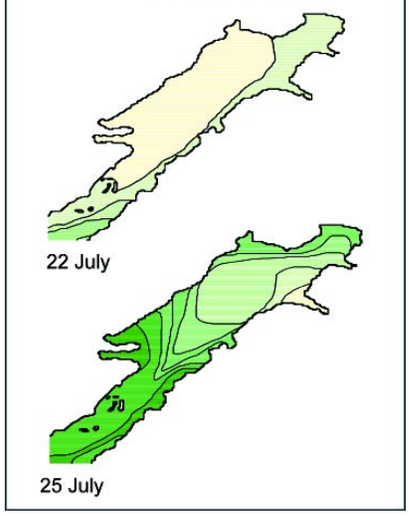
Fig. 7.5
Utermöhl counting technique using a counting chamber and inverted microscope.
(photo: Department of Applied Ecology)

PIGMENT CONCENTRATIONS

- The most generally applicable measure of phytoplankton biomass is the quantification of **chlorophyll a.** However, the extraction procedure, although not expensive, is labour-intensive and time-consuming.
- The measurement of chlorophyll ***in vivo* fluorescence**, a very sensitive and non-destructive method, is a competitive technique (fig. 7.6). The fact that the method can provide information without time-consuming manual pre-treatment of water samples, has stimulated its application. The mapping of chlorophyll concentrations is the most common application of measured *in vivo* fluorescence. For quantitative determinations, *in vivo* data are compared with data on the concentration of extracted chlorophyll a. The production of chlorophyll a distribution maps, on the basis of fluorescence in vivo monitoring, permits the identification of hot spots in a reservoir (e.g., identification of areas where toxic algal blooms form. Box 7.12).

Fig. 7.6
A flow-through fluorometer can be used for monitoring phytoplankton populations in reservoir (photo: M. Izydorczyk)

BOX 7.12
Example of a chlorophyll fluorescence map



INTERPRETATION OF RESULTS

On the basis of the phytoplankton and chlorophyll a concentration data, the trophy of a lake can be calculated. An example of lake classification is provided in the Table 7.4.

TABLE 7.4
Assessment of a lake or reservoir trophy on a basis of various parameters

PARAMETER	OLIGOTROPHIC	MESOTROPHIC	EUTROPIC	HYPERTROPHIC
Phytoplankton biomass [mg L^{-1}]	<0,5	0,5 – 2,5	2,5 - 10	> 10
Average chlorophyll *a* [µg L^{-1}]	<2,5	2,5 - 8	8 - 25	> 25
Chlorophyll *a* (peak concentration) [µg L^{-1}]	4,2	16,1	42,6	> 500

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Guidelines: chapters 1, 2
http://www.algaebase.org/default.html
http://www.nwhc.usgs.gov/pub_metadata/field_manual/chapter_36.pdf
http://www.whoi.edu/redtide/
http://wlapwww.gov.bc.ca/wat/wq/reference/cyanophytes.html
http://www.vcu.edu/cyanonews
http://www.health.gov.au/nhmrc/publications/pdf/eh22.pdf
http://www.vets.org.nz/publicat/vetscript/articles/articlemar03.pdf
http://www.pca.state.mn.us/water/clmp-toxicalgae.html

7.D. WHY ARE CYANOBACTERIAL BLOOMS HARMFUL

One of the most dangerous effects of eutrophication is the formation of phytoplankton blooms, with temporary domination of cyanobacteria during high temperatures and stable hydrological conditions (Codd, 2000). Cyanobacteria can produce different types of toxins, which can cause various health problems or even death if people or animals come into contact with them or ingest them (Ressom et al., 1994; Carmichael, 2001; Chorus, 2001; Falconer 2001;).

The objective of this chapter is to present qualitative and quantitative assessment methods for cyanobacterial toxins.

Fig. 7.7
A Microcystis bloom near the drinking water intake in Sulejow Reservoir, Poland, September 1999 (photo: M. Tarczynska)

WHY ARE CYANOBACTERIAL BLOOMS HARMFUL?

Mass occurrences of cyanobacteria in water cause problems for producing drinking water and for recreational uses of the water.

- the exposure to cyanotoxins is expected to influence both morbidity (ill health) and mortality;
- toxic cyanobacterial blooms can cause health impairments such as skin irritations, allergic responses, mucosa blistering, paralysis of peripheral skeletal muscles and respiratory muscles, hay fever symptoms, diarrhoea, acute gastroenteritis, and liver and kidney damage;
- epidemiological evidence of increased rates of primary liver cancer and colorectal cancer in a specific population in China has been associated with the consumption of cyanobacterially contaminated drinking water (Yu, 1995; Zhou et al., 2000);
- cyanotoxins can accumulate in freshwater mussels, freshwater clams and fish, and transfer through the food chain.

WHAT ARE THE STEPS IN A TOXICITY MONITORING PROGRAMME?

Expensive analytical techniques, modern equipment and high financial support for full chemical analysis of the quantity and quality of cyanobacterial toxins are usually required. A cyanobacterial extract may contain a variety of chemical substances like acids, peptides or pigments, which may be unknown. Their potential effects can only be detected by toxicological testing in conjunction with chemical toxin analysis. In many cases and , more importantly, is an estimation of the toxic effect of complete mixtures, whether they are known or unknown, than detection of selected, individual contaminants.

The first step of investigation should involve bioassays using different organisms (biotest) and enzymes (biochemical methods) for screening the toxicity of complex mixtures. Further, the chemical analysis to determine the quality, quantity and original source of harmful individual substances should be applied (Box 7.13).

The World Health Organization (WHO) recommended 1 microgram per litre as a safety guideline value for the maximum acceptable level of microcystin-LR or its equivalents in drinking water due to the epidemiological character of cyanobacterial toxins (WHO, 1998).

HOW TO DETECT SPECIFIC TOXINS

Biotests (biological methods)

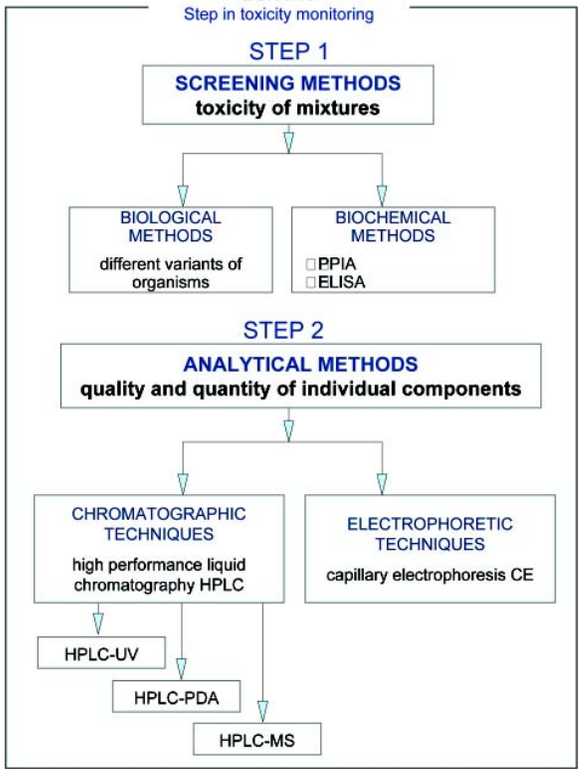
Biotests with different organisms (bioindicators) can provide both estimations of complete mixture toxicity, without determination of the potential effects of individual contaminants (Appendix 6). Synergistic or antagonistic effects between complex mixtures can also be analysed.

In previous investigations, a mouse was the most popular organism that was used to biotest in cyanobacterial research. This kind of bioassay involves oral consumption or intraperitoneal injection

TABLE 7.5
Cyanobacterial toxins and their producers

TOXIN TYPES	CYANOBACTERIAL GENERA
Hepatotoxins Microcystin Nodularin	*Anabaena, Anabaenopsis, Aphanocapsa, Hapalosiphon, Microcystis, Nostoc, Oscillatoria/Planktothrix* *Nodularia*
Neurotoxins Antoxin-a, Homoanatoxin-a Anatoxin-a(s) Saxitoxin	*Anabaena, Aphanizomenon, Cylindrospermum,* *Microcystis, Oscillatoria/Planktothrix, Phormidium* *Anabaena, Oscillatoria/Planktothrix,* *Anabaena, Aphanizomenon, Cylindrospermopsis, Lyngbya*
Cytotoxin Cylindrospermopsin	*Aphanizomenon, Cylindrospermopsis, Umezakia*
Dermatotoxins Debromoaplysiatoxin Lyngbyatoxin Aplysiatoxin	*Lyngbya* *Lyngbya* *Lyngbya, Oscillatoria, Schizothrix*
Lipopolysaccharides (LPS)	Many species of cyanobacteria

BOX 7.13
Step in toxicity monitoring



of a cyanobacterial extract into the mouse and determination of the dose that kills (lethal dose, LD) 50% of mice used in an experiment (LD_{50}). Moreover, biotests with mice provide a characterization of cyanobacterial toxins, which are classified into **hepatotoxins, neurotoxins and toxins with protracted effects.**

Present research needs more ethical, less time-consuming and more cost-effective tests to minimize the use of mammals for experimental testing. Therefore, bioassays for cyanobacterial toxicity require further development of biotests with simple organisms or plants, such as **bacteria, invertebrates, protozoans or plants**.

What conditions have to be met by the organisms used as bioindicators?

For organisms to be widely used as bioindicators a number of conditions have to met (Persoone & Gillett, 1990; Suess, 1982).

- have to be available over the whole year;
- should be genetically uniform;
- have to be healthy and in good condition at the time of testing;
- test reactions have to be evident and repeatable, easy to observe and interpret and have a statistical background;
- in environmental analysis the organisms should be typical of a specific country or region; and
- in heavy contamination conditions they should be sensitive to a broad spectrum of toxic substances.

The sensitivity of these tests with microorganisms or plants should correlate with the sensitivity of vertebrates.

Examples of invertebrates used for testing include *Drosophila melanogaster* and different species of mosquitoes, such as *Culex pipiens*, *Aedes aegypti*, and *Culiseta longiareolata*. Despite the high sensitivity of these organisms, they are not often used because the methods need continuous cultures of test organisms.

Bacterial tests

Bacterial tests as a cost-effective and rapid method are a useful tool for toxicity assessment. Fluorescent bacteria, *Vibrio fisheri*, have been proposed as suitable microorganisms for determining cyanobacterial toxicity (Appendix 6). Bioluminescent tests, such as Microtox, have shown that *Vibrio fisheri* can be used to analyze the toxicity of purified cyanobacterial extracts. ToxAlert, a second toxicity test kit (or toxikit) with Vibrio fisheri, has given positive signals in determining the toxicity of crude and purified cyanobacterial extracts. The other effective bacterial test for determining cyanobacterial toxicity is ToxiChromoPad that uses *Escherichia coli* as the test organism (Appendix 6).

Plant tests

The toxicity of cyanobacterial blooms can be determined using water plants, e.g., *Spirodela oligorrhiza* or *Lemna minor* L. Plants are easier to handle than animals and have proved to be useful in monitoring contamination of water by heavy metals and algal toxins. Cyanobacterial hepatotoxins inhibit the growth of *S. oligorrhiza* by reducing the number of fronds and decreasing chlorophyll (a + b) concentrations.

There is a need to develop new, and evaluate less well known, biotests utilizing higher plants, particularly seeds and seedlings of plants and water macrophytes.

Advantages of water plants (macrophytes) as bioindicators of water pollution

Several authors (Landolt & Kandeler, 1986; Lewis, 1995; Swanson et al., 1991; Wang, 1989) have noted the advantages of using macrophytes as bioindicators of water pollution:

- macrophytes are more sensitive to contaminants than algae;
- they are very sensitive to pesticides;
- they have high reproductive rates (1-4 days), small size and are easier to propagate than other plants;
- water plants (*Lemnaceae*) are more sensitive than invertebrates that were usually used until now;
- easy to use in laboratory conditions; and
- results of laboratory evaluations are comparable to tests conducted in natural conditions.

Advantages of seeds bioindicators of water pollution

The use of seeds as bioindicators produce less problems than fishes, algae and crustacea and their advantages include:

- seeds, typical of a specific climate or environment, are easily available in each geographic location and there are no problems with their transport, storage and preparation for tests;
- seeds can be stored for many years without changes in vigour and physiological condition;

- the physiology and morphology of seeds is well known, which makes their use in bioindicator tests easier;
- seeds of higher plants and their seedlings are sensitive to a broad range of environmental contaminants;
- due to the small dimensions of seeds and their uniformity and low weight, small water samples can be tested;
- test reactions are repeatable and easy to observe, measure and apply;
- for seed tests no expensive equipment is necessary nor is filtration of water samples; and
- seed tests are easy to conduct and are more humanitarian than those on, e.g., fishes.

It is believed that biotests, which make use of seeds and macrophytes, thanks to their low cost, easy procedures and permanent availability of biological material, can play an important role in the bioindication of water pollution, including bioindication of toxic cyanobacterial blooms. Thanks to the high sensitivity of water plants from the Lemnaceae family, information on water toxicity can be obtained within 24 hours.

Toxikits

Quick tests should become an integral part of water quality assessment. To ensure repeatability of results uniform conditions of testing, as well as rearing procedures for test organisms, are essential. In recent years, complete kits for toxicity tests have become available commercially (so-called Toxkits). They include all materials, along with test organisms, necessary for conducting rapid and accurate tests.

Such kits eliminate the problems with the delivery and culturing of enough organisms from the same source in similar conditions. Moreover, organisms in each toxikit have the same sensitivity to toxins. Proper selection of organisms and exposure times enables results to be obtained within a few minutes or hours. The materials and reagents contained in the kit reduce test preparation time and eliminate errors that may occur during reagent preparation. Toxikits guarantee standardization and validation of bioassays. Unfortunately, Toxkits are very expensive and require a high number of replications.

Biochemical methods

Protein phosphatase inhibition assay (PPIA) and **enzyme-linked immunosorbent assay (ELISA)** are rapid (2 hour treatment times) and sensitive screening methods for detection of hepatotoxins and determination of their toxicity (Appendix: 7). Both methods enable detection of very low doses of microcystins, even directly from drinking water supplies below 1 µg L^{-1} microcystin-LR.

A colourimetric protein phosphatase inhibition assay with enzyme protein phosphatase 1 (PP1) and the substrate p-nitrophenylphosphate (p-NPP) is a useful tool for determining the toxicity of microcystins and nodularins contained in cyanobacterial samples.

However, a protein phosphatase inhibition assay, using ^{32}P-radiolabelled phosphorylase as a substrate, has proved to be more sensitive for detecting and determining the toxicity of hepatotoxins than the colourimetric protein phosphatase assay. This radiolabelled assay can use both protein phosphatase 1 and 2A (PP1 and PP2). Unfortunately, equipment requirements for the radiolabelled protein phosphatase assay (e.g., a liquid scintillation counter) are higher than for the colourimetric assay.

Enzyme-linked immunosorbent assay, such as the protein phosphatase inhibition assay, is useful for initial toxicity screening. Commercially the ELISA kit (EnviroGard, EnviroLogix) is available for quantifying microcystins. The estimation of microcystin concentrations by this colourimetric method requires specific antibodies, which are fixed to the walls of tubes or wells in a microplate.

Both the PPIA and ELISA colourimetric methods need a plate reader to measure the results of both the p-nitrophenyl (p-NP) (PPIA) and microcystin-enzyme conjugate (ELISA), assays.

Analytical methods (chemical analysis)

Analytical methods are based on the physical and chemical properties of cyanotoxins, such as molecular weight, chromophores and reactivation products due to the functional groups in the molecules. A summary of chromatographic methods usually used for the detection of cyanotoxins is given in Appendix 8.

The most common analytical procedure for the determination of microcystins (intracellular and extracellular) is **high performance liquid chromatography (HPLC)**, which provides identification of microcystins using their characteristic spectrum at an absorption of 238 nm. The HPLC method can be used for monitoring toxic cyanobacterial blooms in water.

High performance liquid chromatography combined with **UV detection** has been used extensively for the detection of microcystins. But, because this method relies on retention time for identification, microcystin standards are required. Detection by UV can be made more specific by using a photodiode array (PDA) detector, but it has very limited ability to identify individual microcystins because almost all microcystins have a similar UV spectrum (Box 7.14).

To further confirm and identify cyanotoxins analytical methods coupled with **mass spectrometry** are used. For example, liquid chromatography coupled with mass spectrometry (LC-MS) is a very promising method for the simultaneous separation and identification of microcystins in mixtures. Identification of the microcystin characteristic ion at m/z 135, derived from Adda (a unique amino acid, which serves as the key structural component for the biological activities of microcystins), has proven to be useful for discriminating microcystins from other types of compounds.

Also other similar techniques such as **capillary electrophoresis (CE)** and related techniques must also be considered for the separation and quantification of peptide hepatotoxins. CE coupled with mass spectrometry gives a low limit of detection and increased sensitivity method for determining microcystins. However, due to poor result replication this method requires further evaluation.

High performance liquid chromatography, as well as capillary electrophoresis, are the only analytical techniques that can separate and identify cyanotoxins simultaneous (Meriluotoo et al., 1998). Analytical techniques based on either HPLC or LC-MS can also be used for determining saxitioxins, anatoxins or cylindrospermopsin in water.

A physical and chemical screening method that is based on the detection of 2-methyl-3-methoxy-4-phenylbutyric acid (MMPB) as an oxidation product of microcystins, has been reported. Gas chromatography (GC) coupled with mass spectrometry, or HPLC coupled with fluorescence detection, are used to identify microcystin oxidation products.

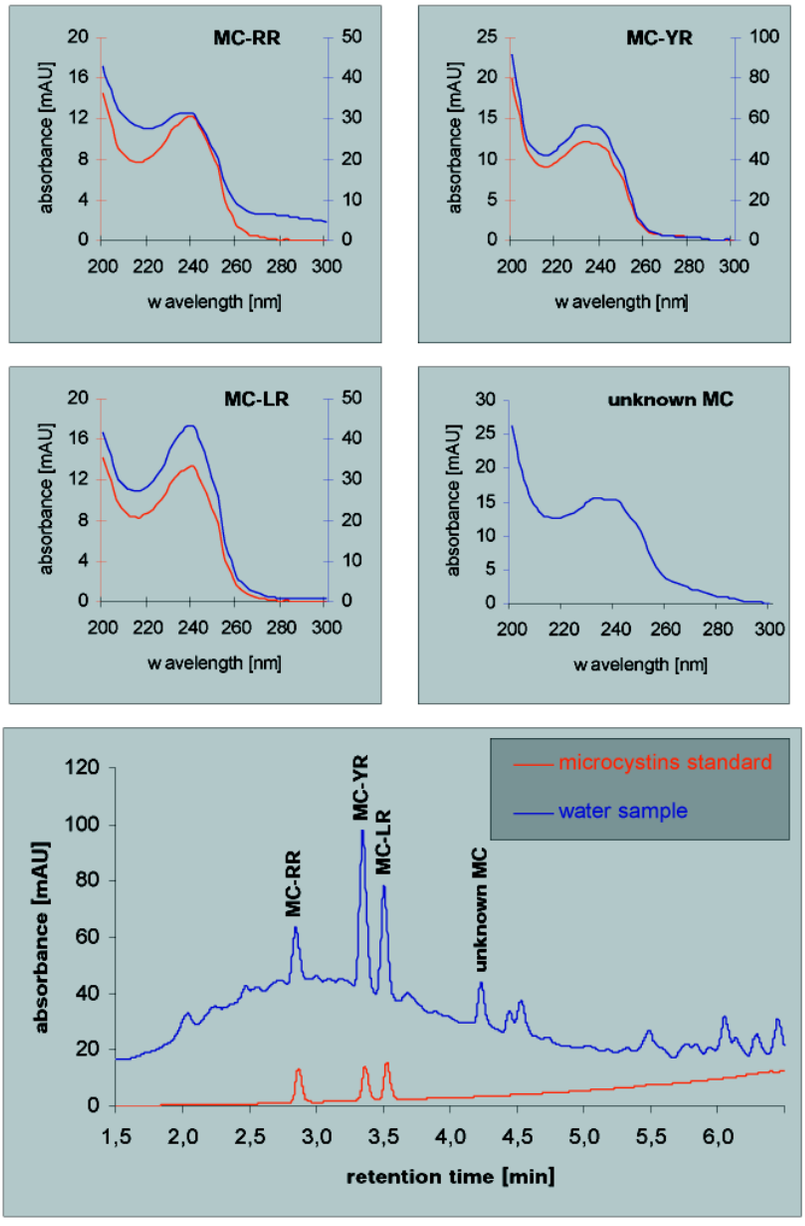
Despite the number of techniques used for identification of microcystins, only high performance liquid chromatography finds application in qualitative and quantitative determinations of cyanotoxins in water and cyanobacterial cells with regards to simplicity, high sensitivity, selectivity and, almost as importantly, high precision. Unfortunately, in situations when standards are commercially unavailable for the majority of toxins the HPLC method cannot be used.

Biological methods of toxin detection (e.g., microbiotest with bacteria and invertebrates) are useful as an initial toxicity screening method for cyanobacterial detection. However, because of low sensitivity and high detection limits for cyanotoxins, they cannot replace biochemical methods (e.g., PPIA and ELISA) or chemical methods (e.g., HPLC).

For the detection of very low concentrations of hepatotoxins, the **PPIA** or **ELISA** test should be used. For qualitative and quantitative chemical analysis of cyanobacterial toxins, HPLC is needed. **Because of our still incomplete knowledge about cyanobacterial toxins and their toxicity, biological, biochemical and chemical methods should be applied jointly.**

BOX 7.14

Chromatogram of microcystins (MC) standard separations and water sample with photodiode array detection and characteristic UV spectrum of microcystins with absorption maximum at 238 nm. Peak identification: MC-RR, MC-YR, MC-LR and unknown peak with typical microcystin spectrum.



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Guidelines: chapters 8

http://www-cyanosite.bio.purdue.edu/

http://www.cyanobacteria-platform.com/main.html

http://www.murraybluegreenalgae.com/

7.E. ASSESSMENT OF ZOOPLANKTON COMMUNITIES

Zooplankton are an important link between phytoplankton primary production and consumers at the higher levels of the trophic cascade (secondary producers). They may impact the pathways of energy flow and matter circulation in freshwater ecosystems.

This chapter presents methods for analysing zooplankton communities in fresh waters. It will also introduce methods for estimating ecosystem status and the ecological state of a lake or reservoir. These take into consideration abiotic and biotic parameters that seasonally influence zooplankton communities.

WHY SHOULD WE INVESTIGATE ZOOPLANKTON COMMUNITIES?

Zooplankton are an important link in the **trophic chain**. They are both omnivores and predators, thus occupying not only the second, but also the third, levels in the grazing food web. Some zooplankton representatives, like ciliates and some Cladocera (ex. *Bosmina* sp.), may also control the microbial food web („microbial loop") as top predators (Lenz, 1992).

The most spectacular role, from the point of view of **water quality** in lakes and reservoirs, is played by larger zooplankters (metazooplankton), which may control phytoplankton blooms. Therefore, understanding zooplankton structure as well as the parameters influencing their community, may be used for manipulation of ecosystem structure in order to allocate nutrients from available to unavailable pools

An additional role that is not often taken into account in cascading interaction studies, is the activity of **predatory zooplankton** such as *Asplanchna* sp. (Rotatoria), *Bythotrephes* sp. or *Leptodora kindtii* (Cladocera). It has been proven that these species have intensive predation rates and can significantly **reduce populations of filtering Cladocera** when the density of planktivorous fish is low (Lunte & Luecke, 1990; Wojtal et al., 1999). Invertebrate predators are very effective and can significantly influence filtering zooplankton populations.

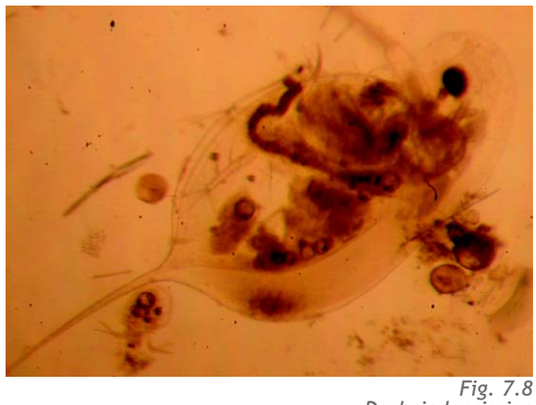


Fig. 7.8
Daphnia longispina
(photo: A. Wojtal)

HOW DO ZOOPLANKTON INFLUENCE WATER QUALITY?

The concept of „biomanipulation" states that zooplankton are the key element in the functioning of most lake and reservoir ecosystems in temperate regions. Whether this is also true for tropical reservoir and lake ecosystems is still unresolved. This is connected with the fact that zooplankton of tropical water bodies are generally of small size and less abundant than in temperate systems. Moreover, to evaluate the functional role of microcrustacean zooplankton in tropical aquatic food webs, it is essential to quantify the dynamics of zooplankton production as microcrustaceans in tropical systems reproduce continuously (Amarasinghe et al., 1997). Our present knowledge recognizes the complex role of zooplankton in aquatic ecosystem trophic structures and their role in exercising top-down control through grazing or predation.

WHAT PARAMETERS INFLUENCE ZOOPLANKTON COMMUNITY STRUCTURE AND DYNAMICS?

Zooplankton community structure and dynamics are regulated by many biotic factors. The most important are:

- abundance and quality of food;
- grazing pressure that releases several defence mechanisms in phytoplankton; and
- fish community structure.

Zooplankton distribution depends mainly on:

- water depth;
- trophic status; and
- water temperature.

An interesting feature of zooplankton is their **daily horizontal and vertical migrations**, which occur at dusk and dawn. Vertical migrations, even up to several hundred metres (*Calanus* sp.), are characteristic of deep lakes, while in shallow lakes, horizontal migration is observed.

Species diversity is generally governed by the **temperature regime**. The highest diversity is found in tropical and subtropical regions and the lowest in extreme environments, such as polar zones and brackish water areas. Temperature is also the most important external factor governing the growth and metabolic rates of zooplankton.

Another significant factor is **food availability**, which varies with season. Thus, the majority of organisms have adapted their life cycle in such a way that they encounter optimal conditions during their reproductive period. Under optimal food conditions, the highest turnover rate is observed in small organisms in the tropics and the lowest in large organisms in polar regions (Harris et al., 2000).

Tropical lakes differ in at least two fundamental properties from temperate lakes: high annual irradiance, and low daily and annual variations in irradiance. These result in a limited number of effects, of which high water temperature, low variation in water temperature and high primary production, are the most important to secondary production (Amarasinghe et al., 1997).

THE ROLE OF HYDROLOGICAL PARAMETERS AND NUTRIENT AVAILABILITY

Important factors regulating the intensity of primary production and, thus food availability for zooplankton, are high phosphorus loads and low N:P ratios. *Daphnia* spp. are known to have much lower C:P (around 80:1 to 100:1) and N:P ratios in their stoichiometry than most other freshwater zooplankton studied so far (Andersen & Hessen, 1991). The high demand for P has two consequences: cladocerans have to minimize P-losses via excretion when algal food is short in P (Elser & Urabe, 1999). This leads to an enhancement of P-limitation of algae. Second, cladocerans might become P-limited if food is abundant but poor in P (Sommer, 1992). The threshold for P-limitation for *Daphnia* seems to be at a food C:P ratio of approx. 300:1 (Sommer & Stibor, 2002). Copepods have a much lower tissue P-content and, consequently, higher C:P and N:P ratios than cladocerans (Andersen & Hessen, 1991).

External nutrient loads to reservoirs depend on catchment geomorphology and use, climatic conditions and consequent hydrological factors of the reservoir and its tributaries. The pattern of tributary discharges also alter abiotic conditions in a reservoir, such as water retention time, water transparency and water column stability. The supply of high matter loads to reservoirs is also important for the development of small filtrator (e.g., *Bosmina* sp.) populations and may regulate the microbial loop.

METHODS OF ZOOPLANKTON COMMUNITY ASSESSMENT

Number and location of stations

The number and location of stations depends on the degree of diversity of a study site.

Stations should be located in sites with different characteristics, which includes hydraulic parameters, water inflow, bottom structure (also presence of submerged macrophytes), depth, physical-chemical factors, predatory pressures, quantity and quality of food (phytoplankton, bacterioplankton, picoplankton), and anthropogenic pressures. For advanced ecological analysis of a water body status, samples should be taken together with water samples for chemistry and phytoplankton biomass assessment (see chapter 7.C).

Sampling methodology

In order to collect zooplankton the following samplers may be used:

- **water bottle samplers**, for taking discrete samples or relatively small volumes of water (a few litres);
- **pumping systems** that sample intermediate volumes of water (tens of litres to tens of cubic metres);
- **nets** of many different shapes and sizes that are towed vertically, horizontally or obliqu-

ely and sample much larger volumes of water (tens to thousands of cubic metres), apply mainly to oceanography studies.

A detailed description of plankton samplers was given by, e.g., Harris et al., (2000).

The type of preservative used to fix zooplankton samples will depend on the purpose for which the samples were taken. In general, zooplankton are preserved in 4% Lugol's solution or in 4% formaldehyde. The various techniques for zooplankton fixation and preservation are given in „Zooplankton fixation and preservation" edited by Steedman (1976).

Time and frequency of sampling

For advanced analysis, zooplankton should be sampled weekly or bimonthly starting at the beginning of the summer season (April-May in temperate regions) up to October, with three replicates for each sampling date.

However, adequate assessment of zooplankton communities can never be conducted on the basis of a single sampling because of the high seasonal fluctuations of zooplankton density and community structure. Therefore, interpretations of the results will very much depend on the degree of understanding of these processes.

UNDERSTANDING SEASONAL ZOOPLANKTON FLUCTUATIONS FOR INTERPRETATION OF RESULTS

A scheme of seasonal fluctuations of phyto- and zooplankton is represented in the PEG Model (Plankton Ecology Group), which was constructed on the basis of the results from 24 eutrophic lakes. In the case of oligotrophic lakes, fluctuations proceed at a slower rate and insom ecosystems do not include all stages (e.g., clear water phase) - Box 7.14.

According to the PEG Model this is a typical specific sequence of plankton succession and includes the following stages (Sommer et al., 1986; Sommer & Stibor, 2002) (Box 7.15):

- **biomass accumulation** during spring -the first phytoplankton biomass maximum due to high biomass of edible phytoplankton (rarely appear in oligotrophic lakes).
- phytoplankton spring bloom is followed by a **clear water phase**, with low phytoplankton biomass caused by zooplankton grazing.
- **decrease of metabolic rate** (decreasing of P:B relationship) of phytoplankton. This process is discontinued during the clear water phase;
- **decrease of zooplankton density is observed** because of enhanced competition resulting from food limitation;
- in summer strong **pressure of invertebrate and vertebrate (fish)** predators may be observed; and
- **biomass decrease of large filterers** (*Daphnia* sp.) may appear in summer because of food limitation. High biomass of inedible algae (e.g., Cyanobacteria).

Clear water phases usually do not occur during summer when there is an abundance of less edible algae that can compensate by growth for the losses of more edible algae. Thus, the top-down impact of zooplankton during summer will mainly be reflected by the taxonomic and size class composition of phytoplankton.

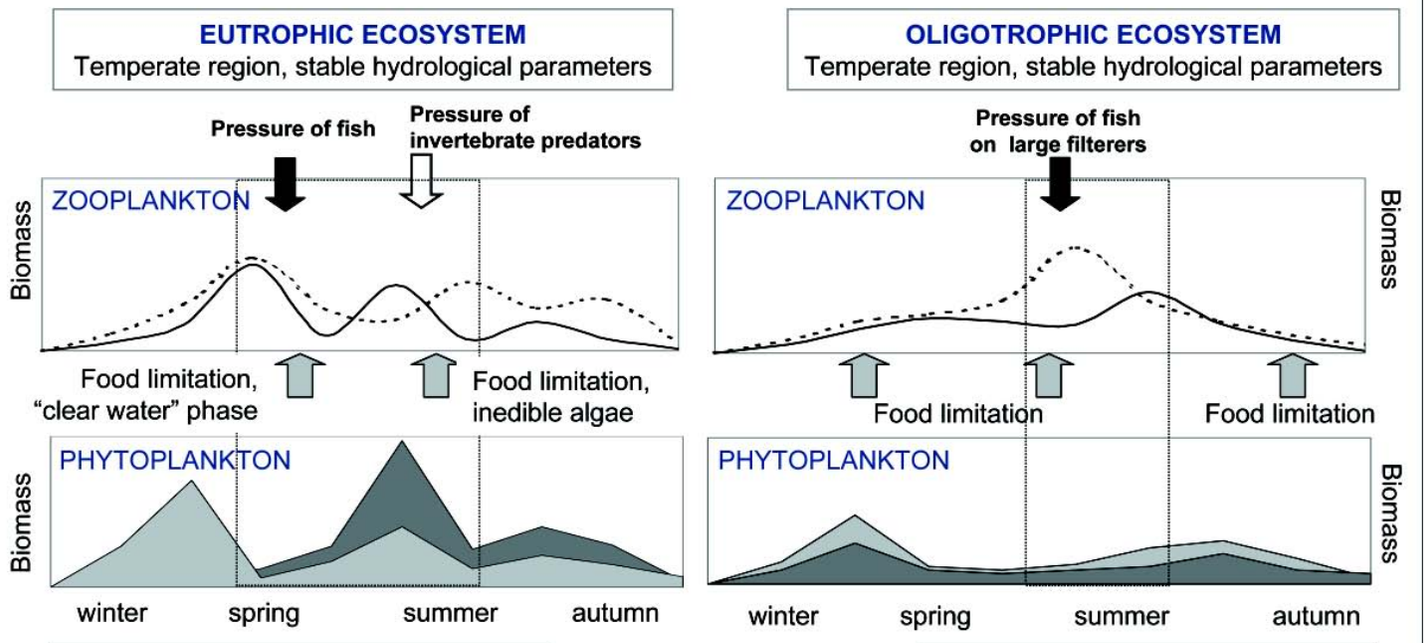
- **switch from phytovorous to detritovorous modes of feeding.** Inedible algae are partly exploited by parasites and detritovorous animals.

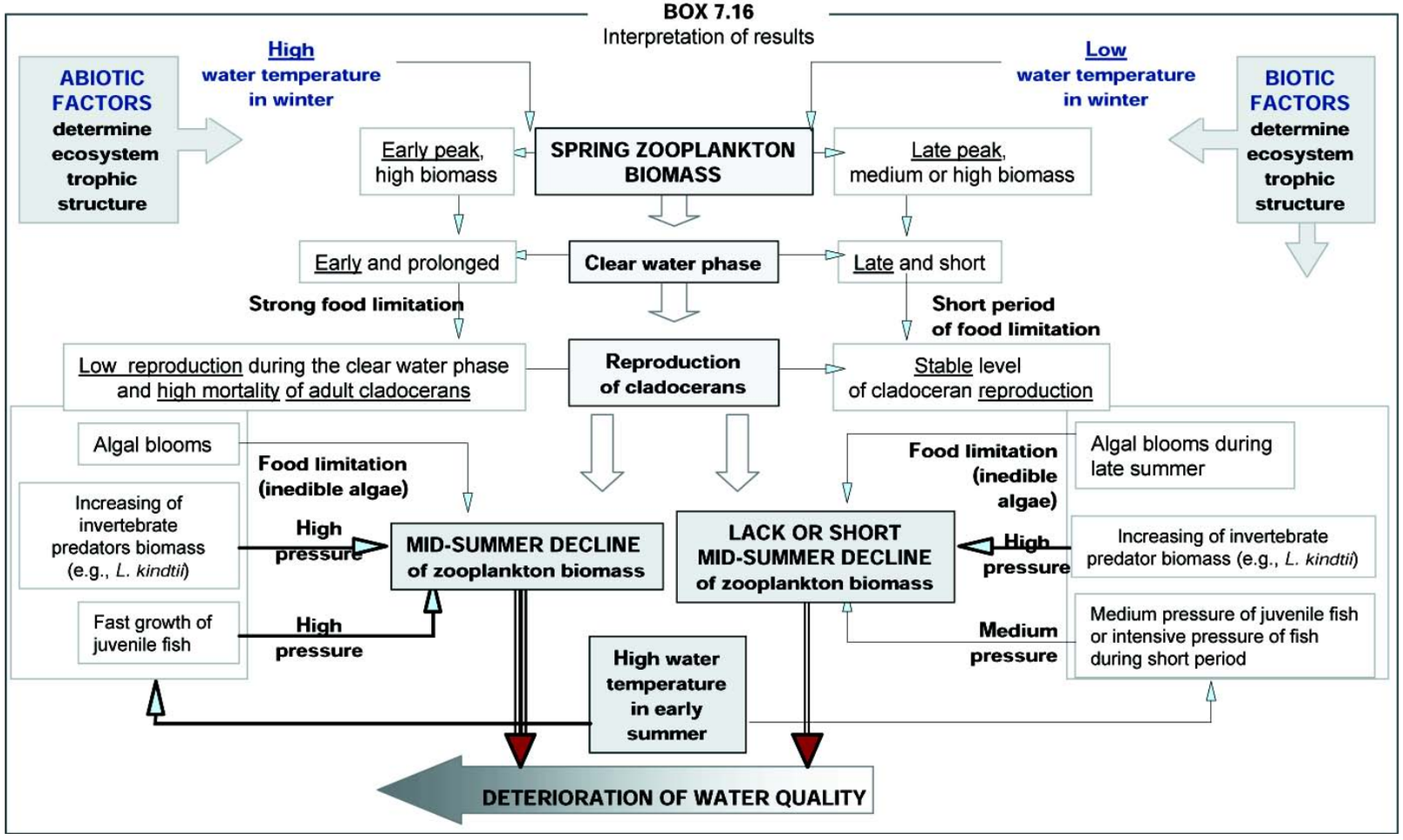
INTERPRETATION OF RESULTS

The schemes presented in Box 7.16 should be helpful in interpretation of your results. It shows the variability of processes in eutrophic lakes where they are mainly dependent on winter temperature, as well as in such systems where processes depend on biotic interactions (mainly predatory pressure). If you find similar results, you should follow the arrows to identify the processes regulating zooplankton communities in your ecosystem.

BOX 7.15

Comparison of zooplankton dynamics in eutrophic and oligotrophic ecosystems





7.F. ASSESSMENT OF FISH COMMUNITIES

The variety of fish responses to changing environmental conditions allows us to assess the state of a freshwater ecosystem on the basis of its fish community structure and abundance of specific fish species. Understanding the fish community structure is essential for decision-making in the process of applying water quality control methods based on ecohydrological relationships, e.g., biomanipulation. The objective of this chapter is to present methods for fish community assessment.

WHAT SAMPLING METHODS ARE USED FOR FISH COMMUNITY STRUCTURE ASSESSMENT?

To achieve a precise picture of fish assemblages, application of various sampling methods adjusted to the characteristics of a given water body, e.g., expected fish species and their age structure, is necessary. The most frequently used quantitative sampling methods for fish communities are:

- **gill netting** - passive capture used for fishing in a pelagic zone. Gill nets have a rectangu lar shape and are usually 50 m long and up to 10 m in width. They are positioned vertically at different depths and regulated by the amount of buoys fixed to the top edge of the net and the weights on the bottom line. To cover all fish age classes, standard gill nets consisting of many sections of different mesh sizes (usually from 5 to 85 millimetres) are used;
- **trawling** or **push netting** - active techniques used for fishing in the pelagic zone (Fig. 7.10). Using these methods one can collect fish from a selected depth. In the case of trawling, the net is pulled behind the boat, while a push net is fixed to the front of a boat. Depending on the power of the boat engine, the length of the net and the size of its opening may differ greatly to enable optimal speed of sampling; and
- **electrofishing** - active capture usually used for collecting both adult and juvenile fish in a vegetated littoral zone. A pulsed D.C. current of 230 V and 3-4 A and an anode equipped with a dip net, are usually used for fish sampling.

Fig. 7.9
Pearch
(photo: www.first-nature.com)

Fig. 7.10
Push netting
(photo: A. Wojtal)

- **beach seining** - active netting used for collecting juvenile fish in a littoral zone (Fig. 7.11). A heavy chain should be fitted to the bottom line of the net to prevent lifting while passing over obstacles. The top edge of the net should float preventing fish escaping over it. While sampling, the net is drawn into the water to form a closed semicircular area. Then, the net is drawn back up, out of the water and on to the bank; and
- **angler interviews** - this method depends on direct counting and identification of fish caught by anglers or/and using data from questionnaires completed by anglers. As angling

is often focused on predatory fish, it is recommended to obtain more complete qualitative data on fish communities by checking what prey-fish species are present in the stomach contents of examined predators.

- in the case of trawling and push netting it is the number of fish captured per cubic metre of net; and
- in the case of beach seining it is the number of fish captured per 100 square metres

Fig. 7.11
Beach seining
(photo: A. Wojtal)

WHAT ARE THE GENERAL RULES FOR FISH SAMPLING?

In order to obtain qualitative data, the following general rules should always be applied to data collection in most lakes and reservoirs:

- sampling should be done at stations **representing the main habitat types** in the water body. It is important to collect fish with different habitat requirements;
- it is recommended to carry out sampling both during the **day and at night** in order to take into account possible data variance due to daily fish migrations; and
- in order to obtain a reliable dataset, sampling should be repeated using the same pro cedure in different seasons of a year, to take into account seasonal fish migrations.

DATA CALCULATIONS

To obtain comparable data, fish catches should be expressed as CPUE (**catch per unit effort**):

- in the case of gill nets it is the number of fish captured during one hour by one square metre of net;
- in the case of electrofishing it is the number of fish stunned by an anode during a given period of time;

WHAT IS MARK-RECAPTURE?

A frequently used method for estimating the abundance of a fish population is mark-recapture. This method is based on collecting fish, marking them, and returning them to a ecosystem. The fish are captured again after a few days and the number of marked specimens is counted. Estimation of total fish number (N) is based on the assumption that the proportion of marked fish in this second sample is the same as the proportion of all marked fish in the total population:

$$N=n_1 n_2/m$$

Where:

n_1 - the number of fish collected and marked in the first catch;

n_2 - the number of fish collected in the second catch; and

m - the number of marked fish found in the second catch.

This method should be used with caution, however, as it requires restrictive assumptions about the population and the marking process:

- marks should be durable and well recognised;
- all fish should be sampled at random;

- marked fish should have the same probability of dying, emigrating and being recaptured as the unmarked ones; and
- during the period of investigation, population abundance should not change.

WHAT ARE HYDROACOUSTICS?

The most sophisticated and accurate tool for estimating fish density and biomass is hydroacoustics (Fig. 7.12). Fish density is calculated as the ratio between the number of targeted fish and the volume of sound-penetrating water. The best results are achieved when fish are randomly distributed and have a low density. Compared with traditional methods of fish community assessment, the use of hydroacoustics has several advantages:

- covers **large areas** within a short time;
- provides huge amount of **data**;
- makes possible **fast computer processing** of data; and
- is **cost-effective** (excluding high initial costs of equipment).

The main weakness of this techniques is lack of species identification and difficulties in acquiring proper data from shallow waters.

Fig. 7.12
Simrad EY 500 portable scientific sounder system
Simrad Company

MAKE SURE TO CHECK THESE RESOURCES:

Guidelines: chapters 8

8.A. WATER CHEMISTRY

Estuaries and adjacent coastal areas are very different in terms of water circulation patterns, morphology, anthropogenic pressures, etc. Thus, general sampling rules are difficult to recommend. In this chapter we refer to the ecological relevance of some chemical parameters, the methods or equipment that can be used, and where and when to collect samples. The reader must critically evaluate the best and most accurate sampling protocols according to his/her sampling site characteristics or study aims.

Fig. 8.1
Water Sampling in coastal areas (photo: L. Chicharo)

WHAT ARE THE KEY PARAMETERS TO BE MEASURED IN COASTAL AREAS?

In estuaries and coastal areas, salinity, dissolved oxygen, pH, turbidity, nutrients and chlorophyll are usually the key parameters responsible for maintenance of adequate conditions for reproduction, growth and survival of species.

Measuring water parameters in estuaries and coastal areas is different from sampling in fresh water because salinity interferes with measurements. In fact, salinity reduces oxygen solubility and increases pH buffering effect. Moreover, turbidity, chlorophyll and nutrients concentrations are lower in saline waters, which requires detection limits to be changed.

Salinity

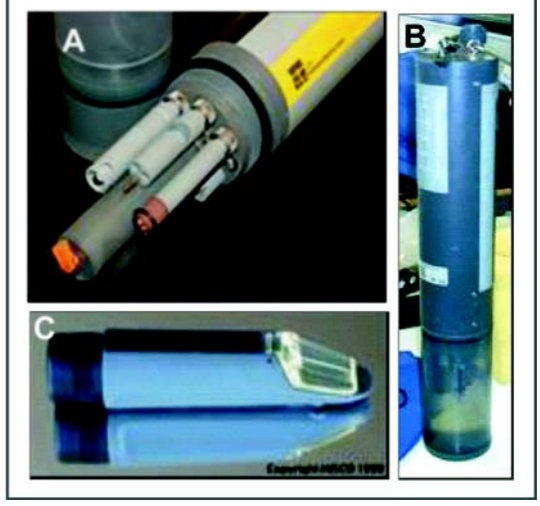
Salinity is a typical parameter measured in order to characterize estuaries and coastal zones. For this reason, particular attention will be placed on this parameter.

Salinity is the concentration of all the salts dissolved in water. The salt in the ocean is mostly made up of the elements sodium (Na) and chlorine (Cl), accounting for 85.7% of the dissolved salt. Together with the other major components of seawater, magnesium (Mg), calcium (Ca), potassium (K) and sulphate (SO_4), they represent 99.4% of the salt in the ocean.

Since water conducts electricity better with increasing salt concentrations, the conductivity of water reflects its salt content (Box 8.1) Salinity can also be measured with a hand held refractometer, but with a lower precision level than with a conductivity metre. When a salinity calculation algorithm is used, results are shown in salinity units and the apparatus is considered to be a salinometre.

BOX 8.1

Sensors used on CTD (Condutivity, Temperature, Depth - A), measuring device - B, Refractometre - C

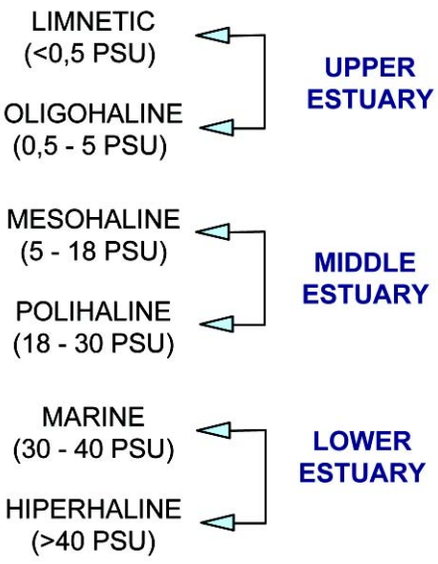


Salinity is usually expressed in practical salinity units (PSU), but also in ppt (parts per trillion) and ‰. More recently salinity is considered without units. The average ocean salinity is 35.

According to the Venice system, Box (8.2), different areas can be delineated in an estuary based on salinity values.

Salinity variations depend on the mixture of fresh water and ocean water. It usually decreases upstream and increases, in a vertical section, towards the bottom. Changes in salinity and water temperature determine water density and influence circulation patterns, allowing the tracking of water circulation in estuaries.

BOX 8.2
Classification of estuarine zones according to the Venice system

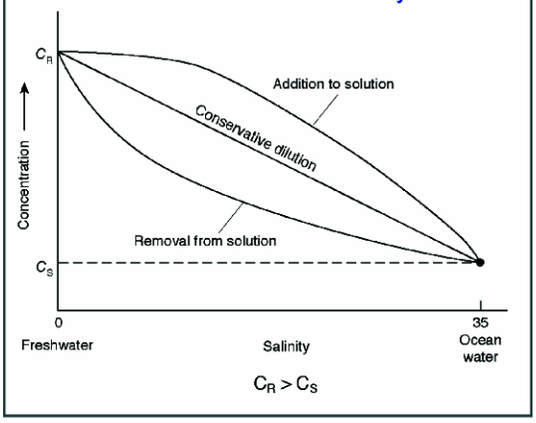


Freshwater discharge affects estuarine ecosystems in a complex way, integrating and linking biological, physical and chemical variables. Generally, fresh water has high contents of Ca^{2+}, SiO_2, Fe, N and P due to chemical weathering or erosion of bedrock and washout of fertilizers or organic waste from land. In contrast, seawater contains high concentrations of electrolytes such as Cl^-, Na^+, SO_4^{2-} and Mg^{2+}. While mixing, salinity behaves conservatively and accordingly has a low involvement in biological and chemical processes. Hence, it is often used as a mixing index. A mixing diagram of conservative material and salinity would show a linear line (Box 8.3).

Concave and convex lines would be observed when a material behaves non-conservatively. A concave line shows the sinking pattern of material according to biological (e.g., photosynthesis) or chemical processes (e.g., adsorption) whereas a convex line indicates addition of material from an estuary that may be created by degradation of organic material or desorption processes.

In coastal areas the influence of oceanographic conditions, e.g., winds, tides and freshwater discharge regimes, are responsible for sudden variations in chemical concentrations in the water, both in time and space. Changes in water chemistry can be indicative of water quality degradation. Ensuring good water quality is fundamental to the maintenance of life and normal uses of estuaries and coastal areas (e.g., recreational, tourism, fishing, etc).

BOX 8.3 [A]
Mixing diagram of material in an estuary



BOX 8.3 [B]
Mixing diagram of material in an estuary

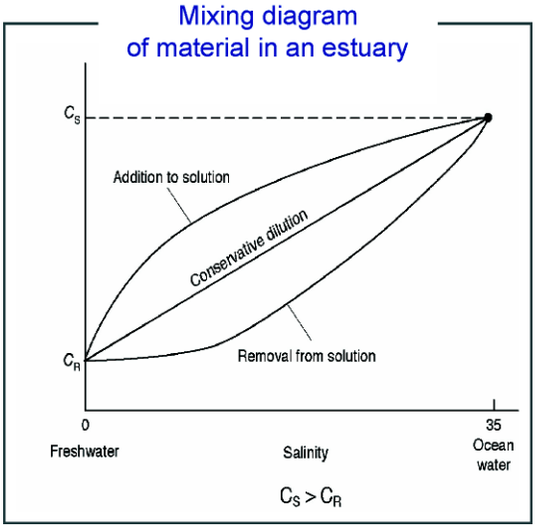


TABLE 8.1

PARAMETERS	UNITS	EQUIPMENT/METHOD NEEDED	WHERE TO SAMPLE	WHEN TO SAMPLE	ECOLOGICAL RELEVANCE
Water temperature	°C	Thermometre Electronic water temperature metre (thermistor)	- Middle channel and river plume - Longitudinal axis: each 1.5 km x 3 depths - At least three depths (surface, bottom, middle)	- Spring and neap tides - Along a tidal cycle - Different Seasons	affects the solubility of many chemicals influences oxygen solubility, water density and occurrence of stratification Information about periodic?? discharges Triggering factor for algal blooms and species reproductive cycles
Salinity	PSU, ‰, none	Refractometre Salinometre Conductivity metre	- Middle channel and river plume - Longitudinal axis: each 1.5 km x 3 depths - At least three depths (surface, bottom, middle)	- Spring and neap tides - Along a tidal cycle - Different Seasons - During exceptional flow regimes	Information about water circulation patterns, Important to species distribution Influence on water properties (e.g., dissolved oxygen or water density)
Dissolved oxygen	%, mg L^{-1}	Electronic oxygen sensor and metre Winkler titration	- Transverse transect between margins - Longitudinal axis: each 1.5 km x 3 depths (summer stratification) - Along estuary channel and subsidiaries - Near potential impact sources (industries, sewers, etc)	- Day/night variation - Different Seasons	Respiratory metabolism of most aquatic organisms Affects the solubility and availability of nutrients Information about bacterial decomposition, Limiting to organisms (0.5-3 mg L^{-1})
pH	None	Colourimetric methods (in non-coloured water, e.g., by algal blooms): - visually - electronically	- Middle channel - Longitudinal axis: each 1.5 km - Transverse transect between margins - Near potential impact sources (industries, sewers, etc) - different depths (summer stratification)	- Day/night variation	Indication of pollution sources Associated with oxygen concentrations (low pH in low oxygen conditions) Solubility determinant of chemicals in the water and, consequently, availability to organisms.

TABLE 8.1 – cont.

PARAMETERS	UNITS	EQUIPMENT/METHOD NEEDED	WHERE TO SAMPLE	WHEN TO SAMPLE	ECOLOGICAL RELEVANCE
Turbidity	NTU (Nephelometric units) Metres (Secchi disk) mg L^{-1}	Secchi disk Turbidimetre	- Middle channel - Longitudinal axis: each 1.5 km - Transverse transect between margins - at ETM (estuarine turbidity maximum) zone	- Spring and neap tides - Along a tidal cycle - Different seasons - During exceptional flow regimes	Influence light penetration and primary productivity (planktonic and benthic) Provides attachment for bacteria and metals
Nutrients (Nitrogen, Phosphorus, Silicon, etc)	µmol L^{-1}	Test Kits/ Colourimeter (not accurate when nutrient levels are low) Spectrophotometer	- Middle channel - Transverse transect between margins - Near potential impact sources (industries, sewers, etc) - different depths (summer stratification)	- weekly-biweekly sampling - end of flow season - peak of high flow season (maximum river water flushing) - several seasons	Influence on primary productivity Development of algal blooms (eventually toxic) Risk of eutrophication (N and P) Changes in Si:N:P ratio (16:1:1) affects phytoplankton succession
Chlorophyll a	µg L^{-1} for plankton species and mg m^{-2} for attached species	Water filtration with a glass fibre filter (posterior determination in a fluorometre or spectrophotometre) In situ fluorometer	- Middle channel - Longitudinal axis: each 1.5 km - Transverse transect between margins - at ETM (estuarine turbidity maximum) zone	- weekly-biweekly sampling - several seasons	Productivity and trophic status of a body of water Indicator of algal blooms

8.B. WATER CIRCULATION

The objective of this chapter is to provide basic information about how to assess water circulation in estuaries and coastal areas using estimations of the current speed, flow rate and residence time.

WHAT IS WATER CIRCULATION?

Water circulation is the result of a complex combination of forces produced by tides, wind and differences in water density.

The most obvious currents in estuaries result from the movement of water caused by tides. Tidal currents often reach their highest speed between high and low tides in the middle of the estuary. Winds also determine the circulation pattern and contribute to the vertical mixing of the water column. The density of water depends on the temperature and the amount of salt **dissolved** in the water. Cold, salty water is denser and warm fresh water is the least dense. When the difference in density prevents mixing between the surface and bottom layers, stratification may occur. Stratification reduces mixing and dilution of materials (e.g., pollutants), and also hampers oxygenation of deeper bottom layers.

Non-tidal currents are caused by the fresh water discharge flow into an estuary and by the resulting differences in densities. In comparison with tidal currents, non-tidal currents move slowly.

Water circulation in estuaries and coastal areas

Fig. 8.2
Guadiana estuary (photo: L. Chcharo)

can be assessed from estimations of current speed, flow rate and residence time.

MEASURING WATER CURRENTS

Current speed may be measured simply by analysing the time necessary for a floating object to travel over a known distance (e.g., between two boats, or two buildings) in a certain direction.

BOX 8.4
Digital flowmetre used to measure flow
and to measure filtered water volume by a plankton net

However, since water circulation may vary with depth due to density differences, it may be necessary also to consider estimations of current speed in deeper layers of the water column. In this case, a normal small bottle filled with 250 ml of water can be suspended with a rope several metres below a surface floating device (e.g., a ball). More accurate results can be obtained by using a current metre. However, current metres are usually expensive. For less accurate determinations a flow metre, as the one used in plankton

nets (Box 8.4), can also be used to estimate current speed. In this case, the current speed (m s^{-1}) can be easily derived from the flow metre readings: FR, number of final rotations; IR, number of initial rotations; and T (in seconds), duration of the immersion from an anchored vessel or quay. From the flow metre a calibration factor, CF, expressed in metres/rotation and indicated in the equipment manual, is used with these variables to calculate current speed:

$$\text{current speed } (ms^{-1}) = ((FR-IR) \times CF)/T$$

BOX 8.5
A stream gauge
used to measure river flow

WHAT IS RIVER INFLOW AND HOW IS IT MEASURED?

An inflow is the flow of water into a stream, lake, reservoir, basin, river, etc. The fresh water input to an estuary or costal zone is measured by the discharge or rate of freshwater flow.

The Discharge or Rate of Flow (RF) is the volume of water flowing through a channel cross-section in unit time (m^3 s^{-1}) (Box 8.5), and can be calculated using the formula:

$$R_F(m^3s^{-1}) = A*(hf(m)-h_0(m))^B$$

Where:

- h_f - final heigth,
- h_0 - initial height,
- A - gauging section - cross-section of the open channel in which depth and velocity measurements are made, and
- B - time between observations (seconds).

WHAT IS RESIDENCE TIME OR FLUSHING RATE?

The flushing rate is defined as the amount of time needed for a parcel of water to travel through a certain part of a river/estuary to the sea and permanently leave a estuary. It is somewhat difficult to measure or calculate the flushing rate of water because there are many factors interfering with the water mass circulation, namely tidal range (i.e., spring or neap), freshwater input and wind speed and direction.

In its simplest form, the flushing time is defined as the time needed to drain a volume, V, through an outlet, A, with current velocity, v. More specifically, the flushing time, t_F, of an estuary can be defined as the time needed to replace its freshwater volume, V_F, at the rate of the net flow through the estuary (the river discharge rate, RF):

$$t_f = V_f/RF$$

Calculation of the flushing time using this method requires knowledge of the volume of the estuary (which is acquired through a detailed depth survey), measurement of the river discharge rate, RF (which can be acquired at a single point at the inner end of the estuary), and a survey of the salinity distribution through the entire estuary.

The observational requirements of a complete survey of the salinity distribution in an estuary can be demanding in time and financial resources. Efforts to derive flushing times from a smaller observational database introduce additional assumptions. The „tidal prism" method starts from the concept that a volume of sea water, V_T, enters an estuary with the rising tide, while a freshwater volume, V_R, enters the estuary during a tidal cycle (rising and falling tides). It assumes that the salt water volume, V_T, is completely mixed with

the freshwater volume, V_R, at high tide, and that the combined volume, V_T+V_R, representing the mixture leaves the estuary during the falling tide. The salinity of the freshwater volume, V_R, is zero. If the salinity of the salt water brought in by the rising tide is S_0, the salinity, S^*, of the mixed water in the volume, $V_T + V_R$, is easily calculated from:

$$(V_T+V_R)S^*=V_TS_0$$

and found to be:

$$S^*=S_0V_T/(V_T+V_R)$$

This gives the freshwater fraction:

$$f^*=(S_0-S^*)/S_0=1-S^*/S_0$$

as:

$$f^*=V_R/(V_T+V_R)$$

The flushing time was previously defined as:

$$t_F=(f^*V)/R_F$$

where:
RF- the river discharge rate or freshwater volume per unit time.

In the tidal prism method the unit of time is the tidal period, T, so RF = V_R / T. Using the result for the freshwater fraction obtained under the assumptions of the tidal prism method:

$$t_F=TV/(V_T+V_R)$$

The combined volume, $V_T + V_R$, represents the difference between high water and low water, therefore often being called the tidal prism. It is the only quantity (besides knowledge of the estuarine volume) required to calculate the flushing time with this method and can be easily obtained from tidal gauge records.
However, the assumptions of the tidal prism method are never completely met in real estuaries. Mixing of the two volumes, V_T and V_R, is never complete and some of the mixed water that leaves the estuary with the ebb tide will enter it again with the rising tide. The flushing time derived from the tidal prism method represents the shortest possible time during which the entire freshwater fraction of an estuary can be removed; in other words, it represents a lower limit for any flushing time calculation.

8.C. STRUCTURE OF BIOTA

Aquatic organisms are very sensitive to changes in the quality of water. They also change in response to a wide variety of pollutants. Thus, individually or in a group (structure and composition of communities), they provide important information about the environmental conditions in which they live, in this case, in estuaries and coastal areas.
The objective of this chapter is to provide basic information about sampling, processing and analysis of biotic components necessary for basic assessment of the biotic structure and composition of estuaries and coastal areas.

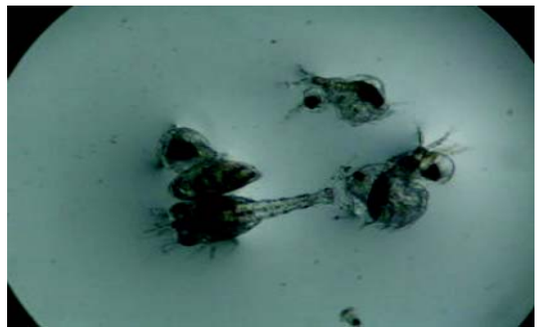


Fig. 8.3
zooplankton
(photo: L. Chicharo)

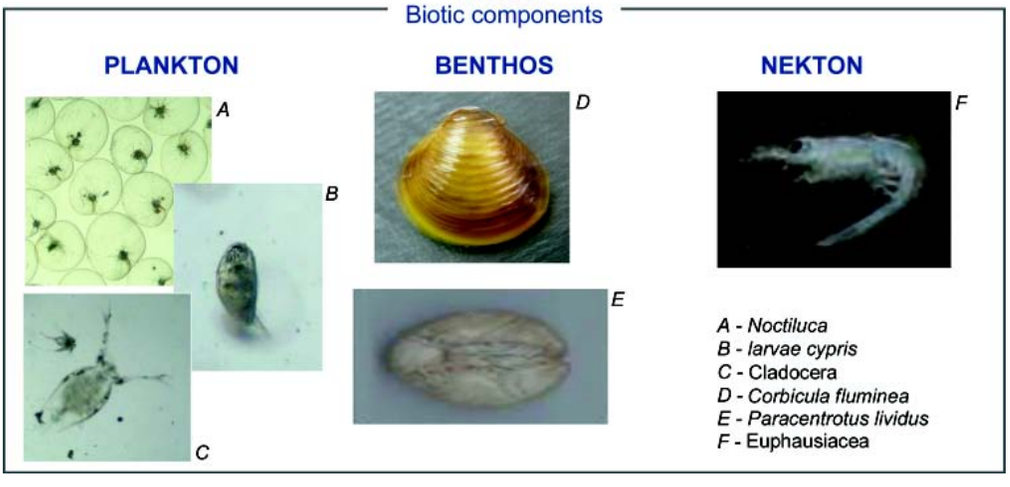
WHAT LEVEL OF ANALYSIS SHOULD BE CONSIDERED IN BIOTIC ASSESSMENTS: INDIVIDUAL OR COMMUNITY LEVELS?

Changes in the biotic structure of estuarine and coastal areas can be assessed based on community or individual analyses. At the community level, usually changes in species abundance and biomass are analyzed. At the individual level, physiological and biochemical characteristics are studied. Studies at the community level have the advantage of providing a global analysis of a system's functioning. However, indicator species (particularly susceptible to certain changes) respond more rapidly to impacts than do communities (except with acute impacts) so that impacts may take a long time to become conspicuous in a community. As a consequence, mitigation and remediation actions are taken only in more advanced stages of disturbance. In contrast, analysis at the individual level rapidly reflects changes in an ecosystem allowing proactive actions to be taken before changes at the community level can be perceived. However, with individual analyses usually only a few species are analyzed and a general understanding of interrelations between species is lost.

Changes at the community level are basically focused on the analysis of species abundance and biomass. Based on a knowledge about the number of individuals per species, several diversity indices (Shannon-Wiener, Pielou, Margalef, evenness, average taxonomic diversity, etc) can be calculated. At the individual level, analysis is focused on the determination of physiological (rates of oxygen consumption and ammonia excretion) and biochemical (RNA/DNA) response to environmental disturbances.

When in the presence of acute impacts that cause sudden and drastic changes in the environment that are responsible for high morbidity and mor-

BOX 8.6
Biotic components



tality rates, conspicuous effects on a particular species or area can be noticed, indicating what and where to sample. In this case, individual sampling could be adequate.

When environmental changes result from long-term disturbance, chronic effects may occur. These are less noticeable than acute effects and usually last long enough to provoke changes in a community.

WHAT BIOTIC COMPONENTS SHOULD BE ANALYZED AND WHAT METHODS SHOULD BE USED?

Selecting the most appropriate biotic components to analyze in estuaries and coastal areas depends on the aims of a study, the type of disturbance, the environmental characteristics in an area and, often decisively, the availability of human and material resources (Box 8.6)

Sampling and processing of estuarine and coastal water samples uses traditional sampling methods for each particular group, but attention must be drawn to the factor of salinity (Box 8.7). In fact, for preservation, conservation or dilutions, osmotic variations may affect organisms, particularly smaller ones, resulting in changes in shape (affecting length measurements) that, in some cases, may cause tissue rupture and loss of biomass. Moreover, pollutants and contaminants may behave differently in the presence of different salinity values, so salinity is a key-factor also for toxicity assessments.

HOW TO ASSESS CHANGES IN STRUCTURE AND COMPOSITION OF BIOLOGICAL COMMUNITIES

Diversity indices

A diversity index is a mathematical measure of species diversity in a community. Diversity indices provide more information about community composition than simple species richness (i.e., the number of species present); they also take the relative abundances of different species into account. Diversity indices (Shannon-Weaver, Margalef, Pielou, Shannon, species richness and eveness) provide important information about the rarity and commonness of species in a community. Results are dependent on sample size and do not reflect phylogenetic diversity. The ability to quantify diversity in this way is an important tool for understanding community structure and changes.

Typically, a decrease in diversity and an increase in species dominance tend to be interpreted as indicative of some type of environmental stress.

BOX 8.7

Plankton and benthos samplers

Vertical plankton net

Bottom dredge

Corer

Grab

Sechi disk

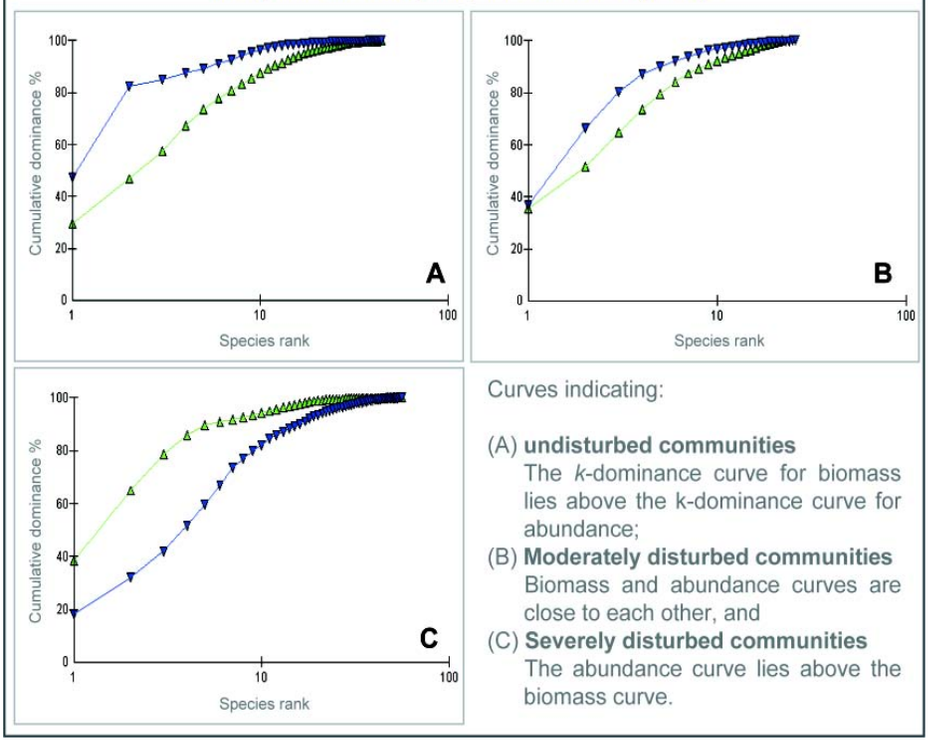
Water sampler

TABLE 8.2
Summary table of sampling, laboratory processing and analysis of different biotic components

BIOTIC COMPONENT	SAMPLING EQUIPMENT AND METHOD	LABORATORY PROCESSING	DATA AND STATISTICAL ANALYSIS	ECOLOGICAL RELEVANCE
Bacteria (coliforms)	3 x 100 ml water samples collected also near sewer and waste treatment discharge points. Collect samples in different tides to evaluate dilution.	Incubation in Petri dishes	Coliform results are reported as Colony Forming Units (CFU) of Total Coliform bacteria counted in 100 ml of water submitted or, Most Probable Number (MPN) per 100 ml of water	The presence of coliform contamination from human or animal wastes indicates water quality degradation.
Phytoplankton	3 x 250 ml water samples collected from bottles (e.g., Van Dorn, Niskin) or pumps	Sedimentation through 10-40 µm mesh filtres	Species composition and abundance Biomass requires calculation of cell volume Diversity indices	Phytoplankton is a Primary Producer and therefore basic to all food webs. Blooms of toxic algae cause water quality degradation.
Zooplankton and icthyoplankton	Nets (mesh size depends on target individual's size) equipped with flowmetre Sampling speed – 2 knots Tow duration – 5-10 minutes, depending on water turbidity (e.g., excessive suspended materials can clog nets)	Sieving and identification Biomass determinations (ash free dry weight, AFDW)	Species composition and abundance Biomass Diversity indices Average taxonomic diversity	Zooplankton and icthyoplankton are first-level consumers and responsible for transference of energy and matter to upper trophic levels.
Benthos	Dredges (e.g., Van Veen) for epifauna and infauna Also, photographs or video images can be used for epifauna Traps can be used for specific studies (e.g., predator-prey relations)	Sieving and identification Individual measurements Biomass determinations (ash free dry weight, AFDW)	Species composition and abundance Biomass Diversity indices Average taxonomic diversity ABC plots (Abundance/Biomass comparison)	Benthic species are a major link in the food chain. Moreover, they remove sediment particles – bioturbation – increasing oxygenation into deeper layers of bottom sediments. Also, they may retain contaminants and pollutants in their bodies, acting as bioaccumulators and bioindicators.
Macroalgae Macrophytes	Dredges Scuba-diving In situ fluorometre	Identification Biomass determinations (ash free dry weight, AFDW)	Species composition and abundance Biomass Diversity indices Average taxonomic diversity	Macroalgae and macrophytes are important reservoirs of nutrients, helping to control eutrophication. However, assessment of limitation by light is necessary. Macroalgal mats are used as nursery areas for several species of fishes.
Nekton	Nets Capture and recapture	Identification Individual measurements Biomass determinations (ash free dry weight, AFDW)	Species composition and abundance Biomass Diversity indices Average taxonomic diversity	Some nektonic species are migratory or use estuaries and coastal areas for reproduction and as nurseries. Nektonic species usually represent the largest part of coastal fisheries.

BOX 8.8

Hypothetical k-dominance curves for species biomass (blue) and abundance (green)

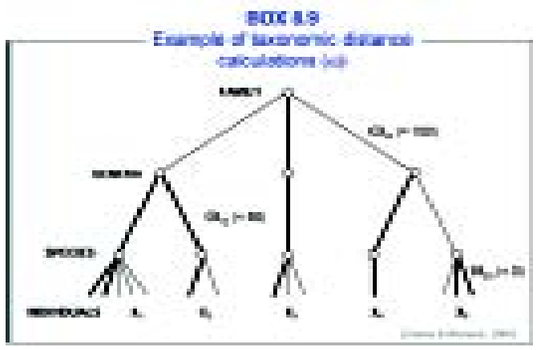


This interpretation may, however, be an over-simplification of the situation. In fact, in situations where disturbance is minimal, the observable decrease in species diversity is caused mainly from competitive species exclusion. However, when disturbance is intermediate, diversity reaches maximal values that usually drop in severe disturbance situations. Thus, diversity indices may indicate the presence of changes but not the level of the impact that cause them (low, medium or high). For this purpose Abundance/Biomass comparison plots (ABC) and the Taxonomic diversity index (Clarke & Warwick, 2001) provide more adequate results.

Abundance/Biomass comparison (ABC) plots

The ABC method involves the plotting of separate k-dominance curves (cumulative ranked abundances plotted against species rank, or log species rank) (Lambshead et al., 1983) for species abundance and species biomass and comparing the shape of the curves (Clarke & Warwick, 2001). Species are ranked in order of importance in terms of abundance or biomass on the x-axis (logarithmic scale) with percentage dominance on the y-axis (cumulative scale). Different types of curves result according to the level of disturbance:

- in **undisturbed communities** the biomass is dominated by one or few larger species, leading to an elevated biomass curve. Each of these species, however, is represented by fewer individuals so they do not dominate the abundance curve, which shows a typical diverse, equitable distribution. Thus, the k-dominance curve for biomass lies above the



k-dominance curve for abundance over its entire length;

- in **moderately disturbed communities** large competitive dominants are eliminated and the inequality in size between the numerical and biomass dominants is reduced so that the biomass and abundance curves are similar;
- in **severely disturbed communities**, communities become increasingly dominated by one or few opportunistic species that, despite their dominant number, do not dominate biomass because they are small-bodied. Hence, the abundance curve lies above the biomass curve throughout its length (Box 8.8).

Taxonomic diversity

One measure, which addresses some of the limitations of diversity indices calculations, is the average taxonomic diversity. This measure, proposed by Warwick & Clarke (1995), considers the taxonomic position of individuals.

Using traditional diversity indices, the same outcome will result from a sample composed of 10 individuals of the same genera or 10 individuals from different genera, but the ecological meaning is different. Biodiversity is, of course, higher in the second case. The average taxonomic change (Δ) of a sample is then defined as the average „taxonomic distance apart" of every pair of individuals in a sample or the expected path length between any two individuals chosen at random (Warwick & Clarke, 1995) - Box 8.9.

Physiological stress indicators

Ecophysiological indices have been widely used to assess changes in physiological conditions of individuals caused by environmental disturbances. Changes in individual condition can be noticed before external evidence of debility and allows estimations of future survival. Therefore, using these indicators it is possible to detect changes that will only cause mortalities after long periods of cumulative impact

Rates of oxygen consumption and ammonia excretion

Studies of the physiology and rates of oxygen consumption (VO_2) and ammonia excretion (VNH_4-N) characterize the energy loss and gain associated with metabolic processes occurring in aquatic individuals. The O:N index, a ratio between oxygen consumption and ammonia excretion rates, indicates the proportion of proteins catabolized for metabolic energy requirements, in relation to lipids or carbohydrates. Therefore, a high protein catabolism compared to lipids or carbohydrates results in a low O:N ratio. Low O:N values have been associated with food limitations (Kreeger & Langdon, 1993). Widdows (1985) demonstrated that O:N<30 indicates the presence of stress factors to mussels.

Biochemical indicators- nucleic acid ratios

Determination of physiological conditions by measurement of the RNA/DNA ratio has been used on a wide range of aquatic organisms (Chícharo & Chícharo, 1995; Chícharo et al., 1998). Organisms in good condition tend to have a higher RNA/DNA ratio and organisms with a RNA/DNA ratio below 1 („minimum ratio") are considered to be in very poor condition with their survival threatened. The use of this index is based on the assumption that the amount of DNA, the primary carrier of genetic information, is stable under changing environmental situations, while the amount of RNA is directly involved in protein synthesis and by inference, with nutritional condition, and therefore more susceptible to negative influences of the environment, e.g., pollution or low prey availability.

RNA/DNA changes have been used successfully in the evaluation of changes in estuarine biota (fish larvae) caused by modifications in river discharge volumes into an estuary. Moreover, in coastal areas this ratio has been demonstrated to be sensitive to changes in oceanographic conditions (changes in currents or presence of upwelling). In fact, Chicharo et al. (1998) and Chicharo et al. (2003) related these factors to the decrease of conditions in sardine larvae and to recruitment failure (Box 8.10).

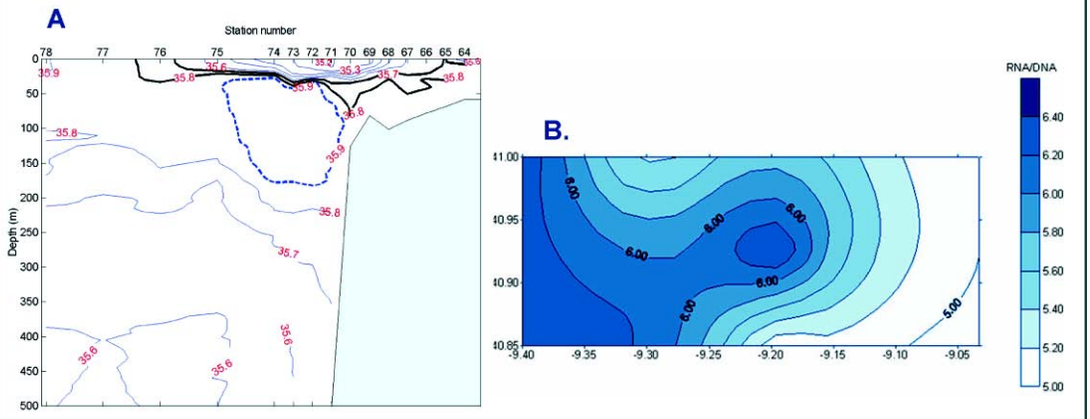
Nutrient ratios

The enrichment of catchment areas in N and P (but not Si) caused by human activities (cultural eutrophication) has been hypothesized as leading

BOX 8.10

A. Vertical distribution of salinity during a winter upwelling event off northern Portugal. Thick solid line represents the Western Iberian Buoyant Plume (WIBP) located over the shelf. River runoff with high values of chlorophyll accumulates here and generates a persistent buoyant plume.

B. Variation of RNA/DNA ratios of sardine larvae reflecting their physiological conditions. High ratios mean that RNA is being synthesized, which indicates cellular growth and therefore reflects good environmental conditions. Physiological conditions of sardine larvae evaluated by RNA/DNA ratios were higher in areas remote from the coast, which was induced by nutrient transport by currents resulting from upwelling.



to a shift from diatom-based to non-diatom-based phytoplankton food webs (cyanobacteria and dinoflagellates), due to exhaustion of Si supplies. The transition of ecosystems from siliceous-based to non-siliceous-based phytoplanktonic communities has been associated with deleterious effects on water quality (Smayda, 1990; Turner & Rabalais, 1994). Redfield et al. (1963) proposed a Si: N: P ration of 16:1:1 as indicating an adequate nutrient ratio for diatom growth. This ratio is within the minimum range for freshwater phytoplankton, since it has been shown that dissolved silicate demand by freshwater diatoms is higher than that by marine species (Paasche, 1980).

Nutrient ratios used to demonstrate potential nutrient limitation are calculated using molar quotients between the in situ concentrations, and delimited by values of Si:N=1, N:P=16 and Si:P=16. These define six different areas, each characterized by potentially limiting nutrients in order of priority, when Si:N, N:P and Si:P ratios are calculated and plotted on an XY logarithmic graph (Rocha et al., 2002) Box 8.11.

BOX 8.11

Nutrient limitation in a water column according to Si:N, N:P and Si:P ratios. In each delimited section, nitrogen (N), phosphorus (P) and silica (Si) are ordered by decreasing degree of limitation