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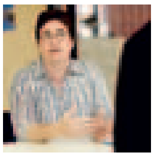
news

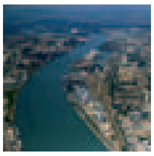
New methods and possibilities in environmental analysis

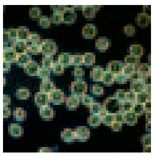
eawag
aquatic research

Content

Focus







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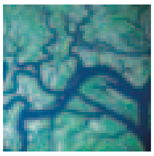

In brief

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Cover photo: Environmental chemist Heinz Singer prepares samples for analysis by liquid chromatography coupled to high-resolution mass spectrometry.
(Photo: Aldo Todaro)

Editorial

Rik Eggen, a molecular biologist, is Deputy Director of Eawag and adjunct Professor at the ETH Zurich.

Increasingly rapid, specific, sensitive and integrated

Recent years have seen dramatic changes in the field of environmental analysis. Advances in genome sequencing, developments in information technology and ever-more sophisticated methods of chemical measurement have opened up new possibilities for research and practice. In both chemical and biological analysis, there is a clear trend towards increasingly rapid, specific and sensitive techniques. In addition, various analytical methods are often combined, permitting a more integrated approach to and understanding of complex systems and relationships.

The current state of the art in environmental chemistry, for example, makes it possible to detect specific substances individually or in mixtures, in different environmental compartments, and at very low concentrations. But the latest analytical methods also allow scientists to detect and determine the possible chemical identities of unknown substances. The introduction of high-resolution mass spectrometry, for example, has transformed chemical analysis – from individual analysis of a limited number of compounds to simultaneous determination of multiple substances with high sensitivity. A much more comprehensive view can thus be obtained of the number, quantities, input pathways and behaviour of chemical substances in the environment. This analytical procedure is making a significant contribution to the development of new, modern concepts for the monitoring of water and surface water quality.

In the past, efforts to determine the effects of chemical substances on organisms were restricted to the assessment of individual biological parameters, such as survival rates. But with modern methods of biological analysis, metabolic functions of organisms can also be determined, and genetic and biochemical processes can be studied in their entirety at the molecular level. Analytical methods in this field are thus becoming more holistic – in the systems biology sense – as they encompass different levels of biological organization. Thanks to recent methodological advances in biological analysis and bioinformatics, researchers now have at their disposal powerful tools for conducting ecotoxicological risk assessments. For this purpose, new standardized tests can frequently be used, which involve cell cultures rather than whole-animal models. Based on these test systems, new effect-monitoring concepts are being developed and implemented in practice.

In its research activities, Eawag is focusing on three overarching topics: "Water for ecosystem function", "Water for human welfare" and "Strategies for trade-offs and competing demands". While the first of these topics involves efforts to gain a better understanding of processes in aquatic systems and the demands of the aquatic environment, the second is concerned with the conditions which need to be met if water is to be used as a resource. Here, modern environmental analysis is ideally equipped to make a vital contribution. The same applies to the resolution of problems and conflicts in practice.

In this issue of Eawag News, we are therefore presenting some of the methods now used at Eawag. And, if you wish to find out more about a particular method or discuss a potential application in your own field, then I would invite you to contact the researchers concerned.

"We need to have better access to environmental data"

Information on the environment is a vital resource for scientific research. And, thanks to advances in information technologies, more observational data is now being collected than ever before. However, as Eawag Director Janet Hering points out, much of this data is not readily available to researchers and practitioners. She is therefore supporting efforts to improve data accessibility.

The amounts of data being gathered today are unprecedented. Our planet is constantly observed by satellites, and monitoring programmes cover almost everything that can be measured – from water pollution to biodiversity. Isn't the information age a paradise for researchers?
It's certainly true that modern information technologies and data collection have opened up possibilities for research which we didn't have in the past. For science and society as a whole, observational data is an essential resource, making it possible for us to reconstruct and understand environmental processes. Long-term observational data, in particular, is needed to identify trends and to establish the effects of human activities on the environment. This information also provides a basis for sound policy decisions. In addition, long-term monitoring is indispensable for assessing the outcome of restoration or mitigation efforts.

What kinds of environmental data are used in aquatic research?
Data on precipitation, river discharge and lake and groundwater levels, as well as on water quality or aquatic biodiversity, are fundamental for our work. And sometimes, scientific questions only arise because of the availability of certain data. For example, it was only historical records of water temperatures in Swiss surface waters that made it possible to investigate the effects of climate change in these systems.

Do any gaps exist in environmental data?
What's more of a problem is the uncertainty about whether long-term data collection will continue. For many environmental questions, long-term monitoring is irreplaceable. Another important point is that information should be kept up to date even after a monitoring programme has been completed. Also, new methodological approaches – such as genetic analysis – should be integrated into existing programmes. But it's not enough just to collect environmental data – the data also need to be freely accessible to researchers and practitioners.

Suitable systems exist, we don't need to reinvent the wheel.

Is that the case in Switzerland?
No, very often it isn't. The Federal Office for the Environment (FOEN), for example, does consolidate cantonal environmental monitoring data, but frequently the accessibility of this data is limited. The data is owned by the cantons and, under the Water Protection Act, only the results of their observations have to be communicated to the federal authorities. Transmission of the actual data is not a legal requirement. In some cases, data accessibility is also complicated by proprietary claims. Many Swiss research institutions also collect long-term data, but only some is passed on to the FOEN. If even just the existing data were freely accessible, that would make researchers' lives much easier.

Is Eawag involved in data collection itself?
In partnership with the FOEN and the Federal Institute for Forest, Snow and Landscape Research (WSL), we run the National

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River Monitoring and Survey programme (NADUF). We are currently also digitally processing data collected over several decades on the occurrence of phytoplankton in five Swiss lakes and adapting the data to modern standards. But compared to the WSL, for example, Eawag has so far been less active in the collection of long-term environmental data.

What could be done to improve data accessibility?
With the Federal Act on Freedom of Information in the Administration, a legal framework for free access is already in place. What's needed now is the willingness on the part of data owners to recognize that, in many cases, the benefits of providing access outweigh individual interests in limiting access – and also justify the costs involved. Next, key data owners would need to agree to cooperate on the development of a shared data management system. Cooperation and joint use of resources would permit an economically acceptable solution. Suitable technologies already exist, so it wouldn't be necessary to develop everything from scratch.

Free access to existing data would make researchers' lives much easier.

A platform of this kind should include all the relevant players – not only the various federal offices, but the cantonal agencies responsible for the collection of environmental data, consulting firms involved in monitoring programmes, and research institutions with experience in data collection and management. Open exchange of data should also be promoted within the Swiss environmental science community.

Have any initial steps been taken in this direction?
Support for freely accessible data is growing. For example, at a workshop organized by the FOEN and the Swiss Hydrological Commission at the end of 2011, the experts agreed that there was an urgent need to improve the availability of environmental information. On the basis of the Federal Geoinformation Act, the FOEN has initiated activities in this area (further information: hugo.aschwanden@bafu.admin.ch). This offers Swiss environmental researchers a good opportunity to promote the scientific utility of easily accessible data. I would certainly welcome cooperation between Eawag and various partners throughout the environmental science community. A partnership with the WSL, based on the data management platform (www.swiss-experiment.ch) established through the Competence Center of the ETH Domain (CCES), is already envisaged (further information: sandro.bischof@wsl.ch).

Key players in environmental observation

Environmental Monitoring Section: Within the FOEN, this section is responsible, in particular, for coordinating collaboration with cantonal agencies in the area of environmental observation. It also manages the Coordinating Organisation for Environmental Observation (IKUB).

Coordinating Organisation for Environmental Observation (IKUB): Represented within the IKUB are the most important producers and users of environmental data from the Federal Administration, the cantons and research. The IKUB coordinates observation activities in various environmental sectors and promotes the sharing of information among key players.

Swiss Environmental Observation Network (NUS): The aim of this network is to develop a coherent nationwide framework for the provision of environmental data. The NUS is currently focusing on the elaboration of geodata models in accordance with the Federal Geoinformation Act.

Envirocat: The Swiss Catalogue of Environmental Data Sources provides an overview of federal and cantonal environmental observation activities, as well as information on available data collections. www.envirocat.ch

Coordinating Agency for Federal Geographical Information (GKG): The coordination of geographical information within the Federal Administration – and of the geo.admin.ch IT platform – is under the strategic direction and management of the GKG. This agency, which includes representatives from all seven Federal Departments and from the Federal Chancellery, is led by the Director of the Federal Office of Topography (swisstopo). www.geo.admin.ch

Coordination, Geo-Information and Services (COGIS): This division of swisstopo is tasked with implementation of the strategy defined by the GKG and is responsible for the development and operation of the Federal Spatial Data Infrastructure.

Intercantonal Geoinformation Coordination (IKGEO): Bringing together cantonal associations responsible for forestry, geoinformation, land registry, agriculture, spatial planning, environment, transport and surveying, this body aims to represent the interests of the cantons vis-à-vis the Confederation.

Sectoral information community (FIG): An FIG is an association of all the players involved in the collection, storage, updating and use of geodata in a particular sector. For example, an FIG has been established for "Waterbodies and Water".

Geocat: The Swiss geographic catalogue provides a gateway for users searching for geodata of specific types (e. g. aerial photographs) or on specific topics (e. g. groundwater, natural hazards). www.geocat.ch

Heinz Singer, environmental chemist, studies the occurrence of micropollutants in natural waters.
Co-authors: Matthias Ruff, Juliane Hollender

Searching for unknown substances

Even at very low concentrations, organic micropollutants in natural waters can be harmful to aquatic organisms. Owing to technical constraints, analytical methods have so far been confined to the detection of a relatively small number of known compounds. With new methods, previously undetected substances can also be identified.

ETH Library, Image Archive

Numerous micropollutants from household, industrial or agricultural sources enter the Rhine (pictured here is the Rhine port at Basel).

More than 65 million substances are currently listed in SciFinder, the world's largest chemical database, with a large proportion being synthetic organic compounds. Around 15,000 substances are added each day, and this rapid growth is likely to continue. In fact, this is not surprising, when one considers that a decillion (= 1 followed by 60 zeros) different chemical structures are theoretically possible. It is difficult to estimate what percentage of the compounds known today are used in significant quantities. An indication is provided by the number of substances which have been notified to the EU since the Regulation on the

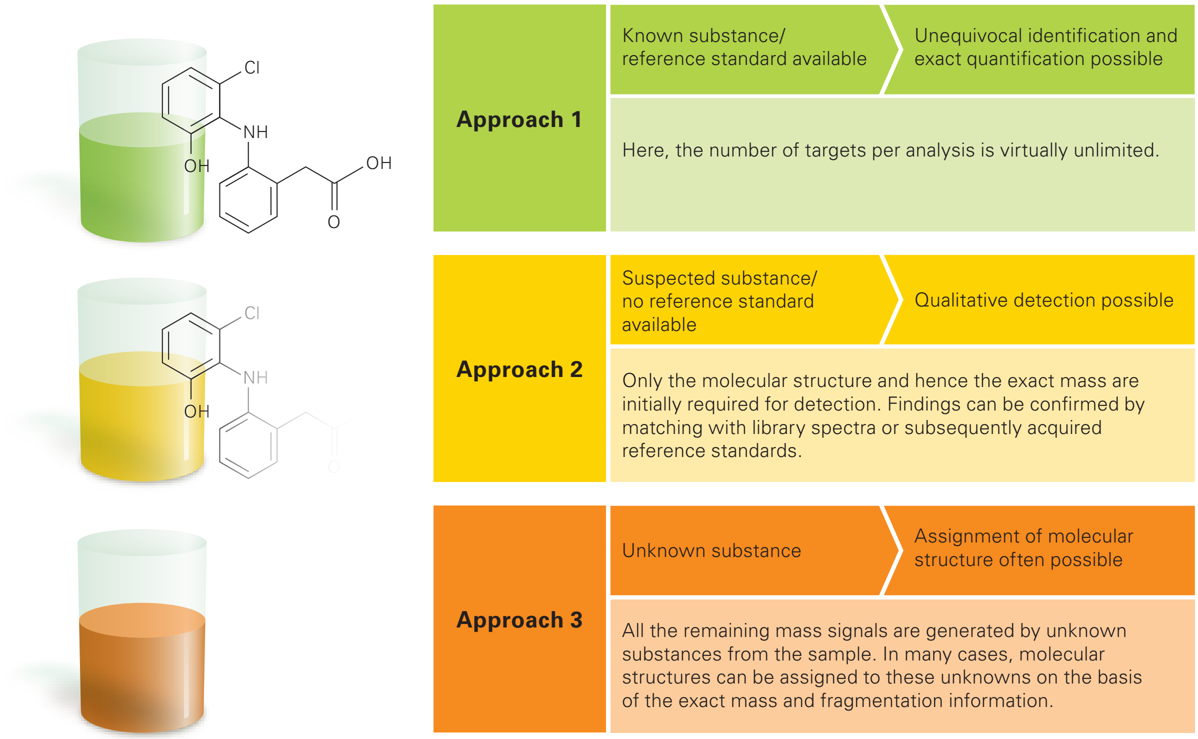


Fig. 1: Analytical and data evaluation approaches for comprehensive screening, using liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) [1].

Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) came into force in 2007: by this reckoning, Europe produces or imports around 140,000 substances.

To what extent – and by what pathways – these compounds enter natural waters can be estimated by modelling. However, reliable predictions can rarely be made, as the necessary information on volumes used, sites of use and substance properties is frequently incomplete. Given the enormous variety of chemicals available, the range of substances which have been evaluated to date and ultimately also detected in waterbodies seems extremely limited, even though it already encompasses several thousand compounds.

More comprehensive analysis possible

In recent decades, research institutes and authorities monitoring wastewater, surface waters and groundwater have detected, in particular, household, agricultural and industrial chemicals, such as pharmaceuticals, pesticides, surfactants, plasticizers and steroid hormones. As more sensitive detection methods have been developed, analyses have become increasingly accurate.

Since the 1990s, gas chromatography coupled to mass spectrometry (see Box, p. 11) has become the method of choice for many non-polar or volatile substances. The coupling of liquid chromatography with tandem mass spectrometry permits the detection of polar and highly hydrophilic compounds. With these methods, however, only a few dozen substances can be studied per analytical run. In addition, the substances have to be selected in advance, and the sensitivity of the detector adjusted accordingly. Under these conditions, one can barely begin to identify the whole range of substances present in a sample.

The situation has been fundamentally changed by the introduction of high-resolution mass spectrometry. With this method, the mass of a molecule can be measured extremely accurately. In addition, it is possible to discriminate between molecules differing in mass by only 0.001 atomic mass units (1.66×10^{-30} kilograms). This means that virtually every substance detected yields a specific signal. The method is also suitable for the entire mass range across which environmental chemicals occur. When combined with liquid chromatography, many of the substances detected can be identified with a high degree of sensitivity. In contrast to previous methods, identification does not require reference standards. These are only needed for purposes of quantification. Essentially, three analytical approaches can be adopted (Fig. 1).

In view of these advantages, liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) could well become established as a standard method for routine surface water monitoring in particular. Apart from the possibility of detecting an almost unlimited number of substances with optimal sensitivity, the highly resolved data can also be used to identify further substances retrospectively. Thus, for example,

Fig. 2: After the chemical spill at Schweizerhalle in 1986, a number of monitoring and sampling stations were set up by the Rhine riparian states.

Fig. 3: At the Weil am Rhein monitoring station near Basel, daily samples are currently analysed for 318 known micropollutants. The aim is to track long-term developments and also to detect problematic discharges as rapidly as possible.

chemicals newly considered relevant to water quality can be extracted from earlier data so as to reconstruct historical water pollution patterns.

Lessons learned from Schweizerhalle

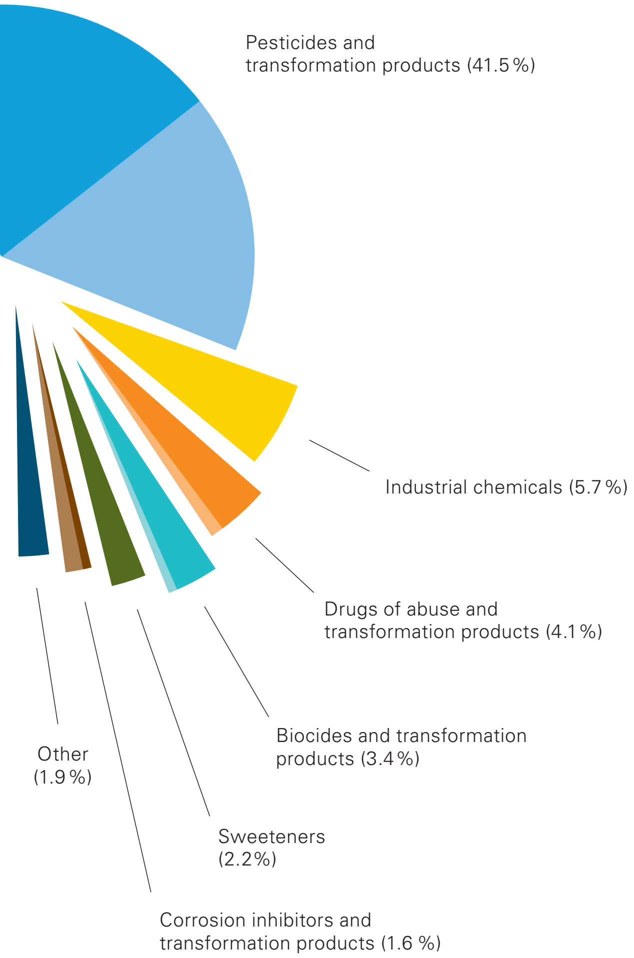
The international monitoring station at Weil am Rhein near Basel has already been using the new method for several years. The Rhine is a source of drinking water for more than 20 million people. Its catchment extends from the Swiss Alps to densely populated regions of Germany and the Netherlands. As well as treated municipal and industrial wastewater, the river receives run-off from intensively used agricultural land and extensive urban areas. The monitoring station began operating in 1992, in the aftermath of the chemical spill which occurred at a Sandoz warehouse at Schweizerhalle in 1986 (Fig. 2). After a major fire, large quantities of toxic chemicals had entered the Rhine with quenching water, causing substantial ecological damage and forcing the temporary closure of downstream waterworks.

Since then, water samples have been analysed daily at the Rhine monitoring station in order to study long-term trends in water quality and also to detect inappropriate or accidental discharges in a timely manner. For this purpose, a multicomponent screening method – based on the LC-HRMS technique – was developed by Eawag in collaboration with the Federal Office for the Environment and the Environment and Energy Office of Canton Basel-Stadt (AUE) [2]. Within the time frame of a day, samples are analysed step by step for known (target), suspected and unknown substances. The list of known substances currently comprises 318 compounds and transformation products from groups including pesticides, biocides, pharmaceuticals, narcotics, industrial chemicals, corrosion inhibitors and sweeteners (Fig. 3). The list is continuously updated with new findings from literature studies and with data from national and international monitoring programmes.

The analyses show that 84 of the 318 compounds from households, industry and agriculture can be regularly detected in the Rhine (Fig. 4). About half of the substances found – with concentrations of less than 10 nanograms per litre – cause barely detectable contamination of the Rhine. A small number of substances exceed the limit of 100 nanograms per litre specified for pesticides in the Water Protection Ordinance. But at this concentration and with a mean discharge of approx. 1000 cubic metres per second, the river is already carrying a daily load of 10 kilograms of each of these substances towards Germany.

Identifying unknown substances

Although the analysis is extremely comprehensive compared with previous methods, it remains unclear what proportion of



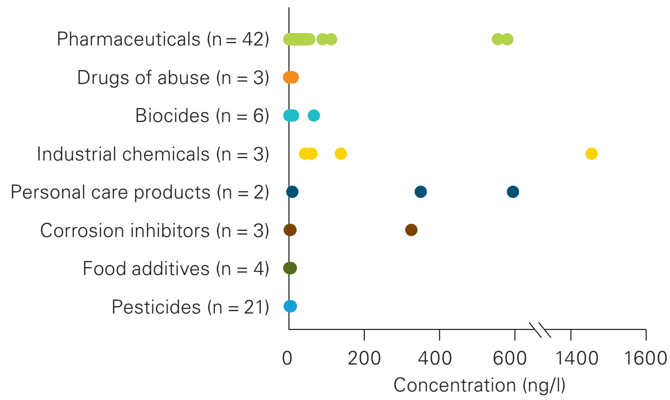


Fig. 4: 84 household, industrial and agricultural chemicals can be regularly detected in the Rhine.

the substances present in the Rhine is actually detected. In order to screen for unknown substances, Eawag has developed software which can extract all the substance signals from the mass spectrometry data, sort them by intensity and eliminate background signals. Evaluation of the data reveals that, as well as the quantified micropollutants, a sample contains on average between 2000 and 3000 other compounds. Of these, 100–150 show signal intensities which suggest a concentration of more than 10 nanograms per litre. Thus, numerous organic compounds have previously gone undetected in the Rhine. Whether these are synthetic or naturally occurring substances remains to be established.

For unknown substances of this kind, no information is available apart from the analytical data, such as the precise molecular mass or isotope pattern. The identification of suspected substances therefore has to proceed in accordance with Approach 2 (Fig. 1). In the absence of reference standards, one searches within the water-relevant group of pesticides, biocides and pharmaceuticals for registered active substances with an appropriate molecular mass. If necessary, hits can be confirmed with the aid of spectral libraries.

In most cases, however, unknown substance masses have to undergo elaborate evaluation (Approach 3). On the basis of the precise mass and the isotope pattern, an unequivocal molecular formula can first be assigned to each substance. Matching structures can then be sought in specialized databases. Finally, comparison of the fragmentation patterns leads to the most likely structure. Using this approach, we were able, for example, to successfully detect the antidepressant moclobemide. Between 12 and 28 January 2011, a chemical suddenly appeared in the Rhine samples in the form of a strong mass signal, which was assigned the structural formula $C_{13}H_{17}Cl_1N_2O_2$ (Fig. 5). A measurement campaign conducted along the entire length of the Rhine during a second discharge period from 9 to 15 March 2011 clearly indicated a point source lying between Rheinfelden and Basel. Assessments showed that about 14 kilograms of moclobemide entered the river during this period, and around 40 kilograms in January. The substance could be tracked all the way to the river's mouth in the North Sea. It was calculated that the moclobemide concentrations detected did not pose a significant risk to the Rhine or to drinking water produced by bank filtration.

The LC-HRMS method can readily be used to detect accidental or inappropriate discharges. As the parties responsible often do not notice such accidents or are unaware of the precise composition of the effluents discharged, daily monitoring can detect contamination peaks in the Rhine in good time and ideally also

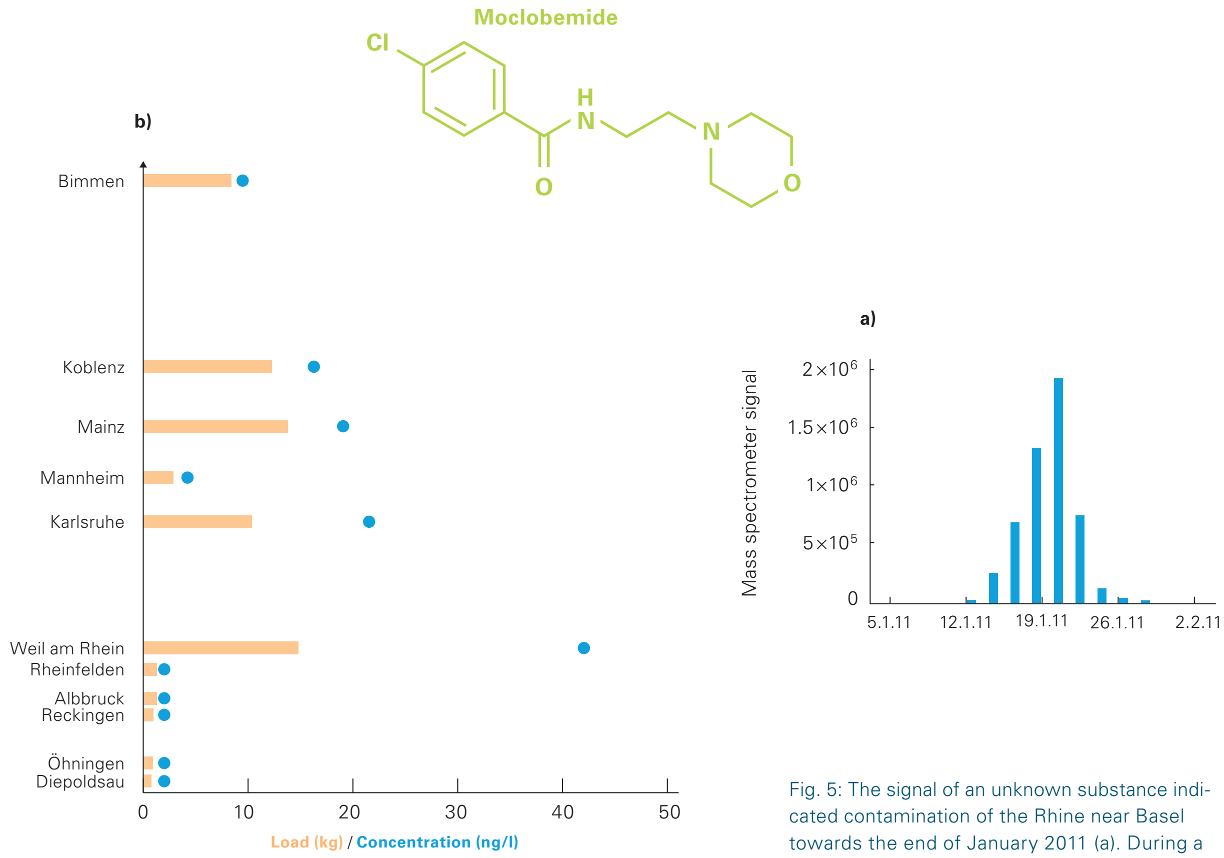


Fig. 5: The signal of an unknown substance indicated contamination of the Rhine near Basel towards the end of January 2011 (a). During a second discharge period, the substance was measured all the way from Switzerland to the Netherlands (b).

identify the substances in question. At present, however, the identification procedure is still too time-consuming for routine daily use. As the next step, therefore, the detection of substance masses appearing unexpectedly is to be integrated into the monitoring station's daily operations with the aid of statistical evaluation methods.

Detecting unknown transformation products

Because of the costs involved, monitoring of organic micropollutants in large catchments and at the national level generally only covers selected priority substances. To review the selection of substances, Eawag carried out several pilot studies using the LC-HRMS method, in cooperation with the federal and cantonal authorities. Analyses were conducted within the national groundwater (NAQUA) and surface water (NAWA) quality monitoring programmes, and also in Lakes Constance and Lucerne. Measurements were combined with predictive exposure models. It was thus possible, for example, to predict exposure for the Lake Constance catchment using a material flux model and, in addition, to measure the catchment-specific substances at selected sites [3].

The fact that this approach can also be effective in extremely large catchments such as the Haihe river system in Northeastern China is demonstrated by the results of a joint international research project (cf. the article on p. 36). This river system extends over an area of 300,000 square kilometres. As more than 20 million people live and work in this region, it is extremely difficult to predict what organic micropollutants will be found. Using LC-HRMS analyses, the relevant substances were detected with only a small number of samples. These priority substances were then studied to determine input pathways and their fate in the catchment area. It was found that the wastewater treatment plants of Beijing are the main source of organic micropollutants. As over 90 per cent of the water downstream is used for irrigation, the organic micropollutants also find their way onto the agricultural fields [4].

Apart from its use in water quality monitoring, high-resolution mass spectrometry can also provide important additional information in laboratory tests. For example, in biodegradation experiments, Eawag scientists detected previously unreported transformation products of pharmaceuticals in wastewater [5]. Possible transformation products were first predicted with the aid of a computerized expert system. LC-HRMS was then used to screen for the suspected structures in wastewater. In some cases, it was possible to confirm the identity of the compounds by comparison with reference standards in samples from wastewater treatment plants [6].

The above examples from research and practice reveal the considerable potential of the new analytical technique. However,

Raoul Schaffner

this can only be fully exploited if continued efforts are made to develop efficient software routines suitable for automation. As in other data-intensive fields, success is largely dependent on advances in data analysis. The combination of mass spectrometry methods with appropriate computer-aided evaluation algorithms is essential if this technique is to attain its full potential in the future.

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Detection methods

Chromatography is used to separate a mixture of compounds into its individual components. In this technique, the mixture – transported in a mobile phase – is passed through a separation column with a stationary phase (modified silica gel particles or coated capillary). Depending on their physical properties, the individual components are more or less strongly retained on the stationary phase and are thus separated. The substances successively eluting from the column are measured as electrical signals (peaks) by a detector. They can then be identified by comparison with the curves for reference standards (calibration curves). The substances can be quantified on the basis of the peak intensity or area. In **gas chromatography,** gas (generally helium or hydrogen) is used as the mobile phase; in **liquid chromatography,** liquids (mixtures of organic solvents or water) are used for this purpose. Thus, with gas chromatography only vaporizable substances can be analysed, whereas with liquid chromatography all soluble compounds can be separated. Because of their selectivity and sensitivity, mass spectrometers have become the detectors of choice.

With **mass spectrometry** the mass of a molecule can be determined. After chromatographic separation, the substances to be analysed are ionized in an interface. In the mass spectrometer, the ions are first separated according to their mass-to-charge ratio in an electric or magnetic field and then detected. As well as intact molecules, fragments are generated whose size and abundance can be used for the identification of substances. In **tandem mass spectrometry,** two instruments are combined in sequence so as to enhance selectivity and sensitivity. With a **high-resolution mass spectrometer,** ion masses can be measured with high accuracy, making it possible to discriminate between ions with very similar mass-to-charge ratios. This is useful in elucidating the structure of unknown compounds.

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Co-authors: Reto Wijker, Stephanie Spahr

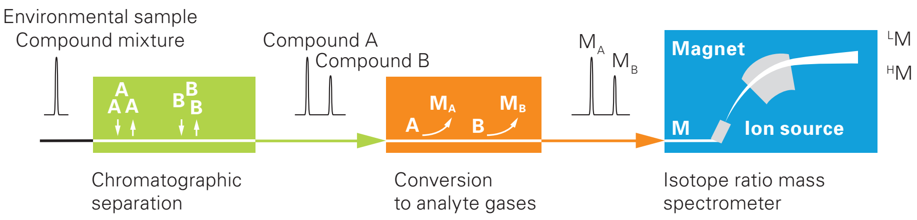
Isotopes: tracking pollutant sources and breakdown

The fate of organic micropollutants in soils and natural waters is difficult to track using traditional methods. But with compound-specific isotope analysis, the isotopic composition of contaminants can be studied. This makes it possible, for example, to trace the origin of dishwasher detergents or to determine the degradation pathways of explosives.

Reto Wijker

Remediation of a contaminated site formerly used by a Swiss explosives manufacturer: with the aid of isotope analysis, degradation processes in the subsurface can be reconstructed.

In assessing the quality of natural waters, it is essential to take organic micropollutants into account [1]. Tens of thousands of chemicals are used in industry, commerce and households. In spite of wastewater treatment efforts, many of these substances end up in surface waters, where they can cause adverse effects even at low concentrations. In addition, organic contaminants are released from landfills and contaminated sites. As concentrations of these substances often exceed those in



Schematic view of compound-specific isotope analysis (adapted from [2]). After being separated by chromatography, individual compounds are converted into a measurement gas (M) suitable for analysis. An isotope ratio mass spectrometer simultaneously measures two ion streams of light (abundant, ^{L}M) and heavy (rare, ^{H}M) isotopologues.

soils and waters, contaminant immobilization or site remediation measures need to be considered. In deciding whether action is required in practice, the following questions are particularly relevant: Where did the pollutants originate, and who might be responsible for their release into the environment? If transformation processes occur, do they lead to toxic or benign products?

More accurate picture of sources and degradation

With modern analytical methods such as liquid chromatography coupled to high-resolution mass spectrometry (see the article on page 6), trace organic contaminants can be identified and their concentrations quantified. However, identifying the source becomes challenging if various emitters could be responsible. And decreasing concentrations do not necessarily indicate transformation, as such observations may be due to dilution, sorption of micropollutants to particles and sediments, volatilization or degradation. Determining if and to what extent contaminants are typically degraded requires labour-intensive measurements supported by modelling efforts. In addition, transformation products may also be problematic in terms of ecotoxicity and should be included in risk assessment.

Using compound-specific isotope analysis, researchers at Eawag are developing new approaches for determining the source of organic contaminants and characterizing transformation processes. With compound-specific isotope analysis, stable isotope ratios can be measured for individual compounds – typically carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$) and hydrogen ($^{2}H/^{1}H$) (see box). First, however, the compounds in an environmental sample need to be separated chromatographically from other constituents. This is generally done by gas chromatography coupled to isotope ratio mass spectrometry. Intensive research efforts are currently under way to expand the range of applicable separation methods (e.g. liquid chromatography) and elements amenable to isotope analysis, such as chlorine ($^{37}Cl/^{35}Cl$) and bromine ($^{81}Br/^{79}Br$) [2].

To illustrate the application of compound-specific isotope analysis (CSIA), we discuss two examples of recent Eawag research.

Compound-specific stable isotope analysis

Organic compounds contain a variety of isotopic elements and thus numerous isotopologues (molecules differing only in their isotopic composition). It is therefore not possible to determine isotope ratios directly from the molecular mass with sufficient precision using conventional mass spectrometry.

In **compound-specific isotope analysis** (CSIA), to solve this problem, each compound is converted into a measurement gas of low mass, in which only one or two atoms of the element to be analysed (analyte) are present. In an isotope ratio mass spectrometer (IRMS), the isotopes of the analyte can be simultaneously measured as two separate ion streams, containing the heavy (rare) and the light (abundant) isotopologue. For example, the carbon skeleton of a contaminant is oxidized to CO_2 at around 1000 °C and quantified in the mass spectrometer as the ratio of $^{13}CO_2$ to $^{12}CO_2$. To analyse nitrogen, hydrogen and oxygen isotopes, N_2, H_2 and CO are used as the analyte gases.

The measurement of an isotope ratio carried out in this way is extremely precise even if the abundances of the isotopes differ markedly (^{13}C makes up only 1.1 % of the element carbon, and ^{2}H only 0.015 % of hydrogen). However, the method is only applicable for elements which can be continuously converted to analyte gases.

In addition, because isotope measurements vary from one instrument to another, measured isotope ratios always have to be normalized to standard reference materials. The result is the **isotope signature** given as a δ value, which expresses the per mil (‰) difference between the isotope ratio in the sample and that in the reference material. In the case of carbon isotope signatures (δ13C), for example, the following equation applies:

$$\delta^{13}C\ (‰) = \frac{^{13}C/^{12}C\ (\text{sample})}{^{13}C/^{12}C\ (\text{reference})} - 1$$

With gas chromatography coupled to isotope ratio mass spectrometry, the typical measurement uncertainty for carbon isotopes is ±0.5 ‰. For two measurements, this corresponds to a difference in the $^{13}C/^{12}C$ isotope ratio of only 0.0000056.

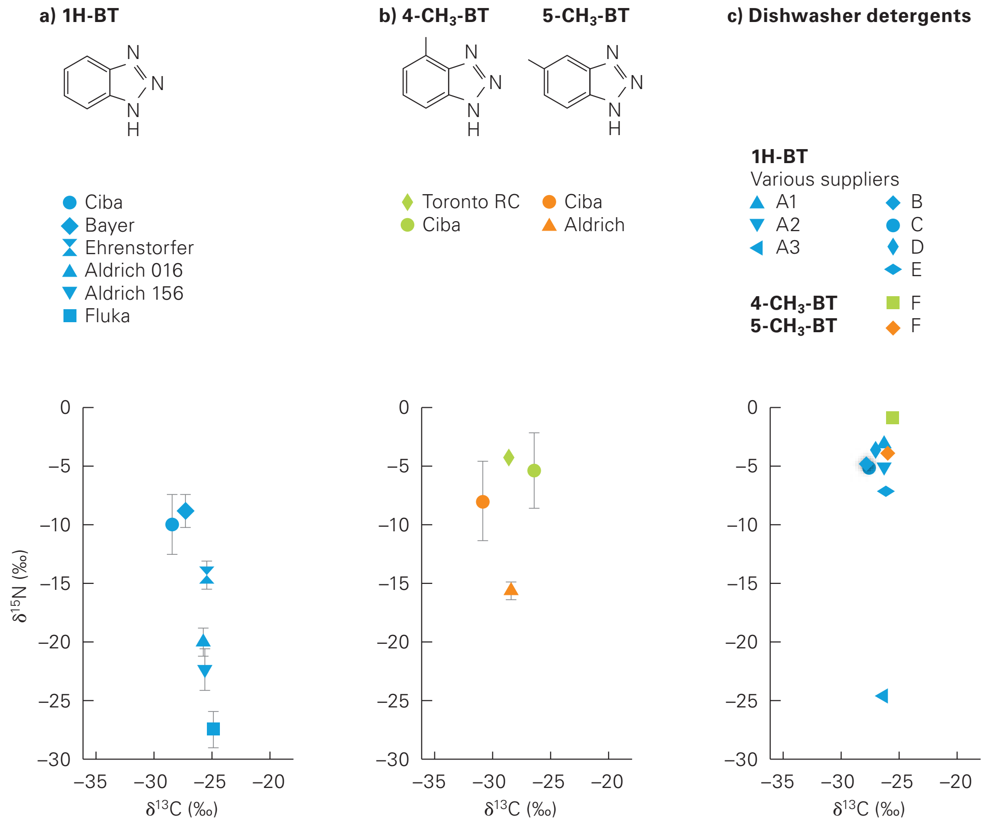


Fig. 1: Carbon and nitrogen isotope signatures of benzotriazole (1H-BT) and two methylbenzotriazoles (4-CH_3-BT, 5-CH_3-BT) in chemicals from various suppliers. Shown on the right are the $\delta^{13}C$ and $\delta^{15}N$ values for the same substances in dishwasher detergents from various suppliers (adapted from [6]).

As is shown for benzotriazoles, similar isotope ratios are indicative of identical production processes and raw materials, thus permitting identification of the source. Varying isotope ratios, however, as observed for nitroaromatic compounds at a site contaminated with explosives, provide evidence of transformation processes and products, and also – with additional information – rates of degradation[3].

The origin of benzotriazoles in dishwasher detergents

Benzotriazoles are among the most frequently detected organic micropollutants in Swiss waters because they are used in large quantities in industrial and consumer products such as corrosion inhibitors and antifreeze agents [4]. They serve as indicators of persistent organic pollutants and are used to assess the performance of wastewater treatment plants (WWTPs) [5]. Whether and under what conditions benzotriazoles (and presumably also other micropollutants) in surface waters can be biodegraded at WWTPs could be determined with the aid of CSIA. This would require the detection of changes in the composition of the carbon or nitrogen isotopes in benzotriazoles. However, because of their tendency to form complexes with metals, benzotriazoles cannot be measured by the conventional method of gas chromatography coupled to isotope ratio mass spectrometry. At Eawag, we have therefore developed an alternative approach for CSIA of such polar organic micropollutants. On this basis, we tentatively assigned benzotriazoles in dishwasher detergents to possible suppliers.

Isotope analysis involves comparison of the very different signal intensities of heavy and light isotopes, and thus requires high contaminant concentrations. Our newly developed approach, using nickel-platinum reactors to convert benzotriazoles to CO_2 and N_2, requires 22 nanograms of the element carbon and 11 nanograms of nitrogen for accurate measurement. In a microlitre injection, this is equivalent to a benzotriazole concentration of 30 – 40 milligrams per litre. While such quantities are relatively low for CSIA, the concentrations are 10,000 times higher than those typically observed in surface waters or WWTP effluents. For this reason, it is necessary to enrich benzotriazoles from environmental samples using solid-phase extraction, without altering the isotopic composition of the target compounds. It is then possible to determine the C and N isotope signatures of benzotriazole (1H-BT) and methylbenzotriazoles (4-CH_3-BT and 5-CH_3-BT) in tap water, WWTP effluents, sewage sludge and dishwasher detergents by gas chromatography coupled to isotope ratio mass spectrometry.

Isotopic fingerprint determined by chemical production

Figures 1a and b show the $\delta^{13}C$ and $\delta^{15}N$ values of benzotriazoles from various chemical suppliers. It is noticeable that the

Remnants of TNT and DNT at the contaminated site.

Reto Wijker

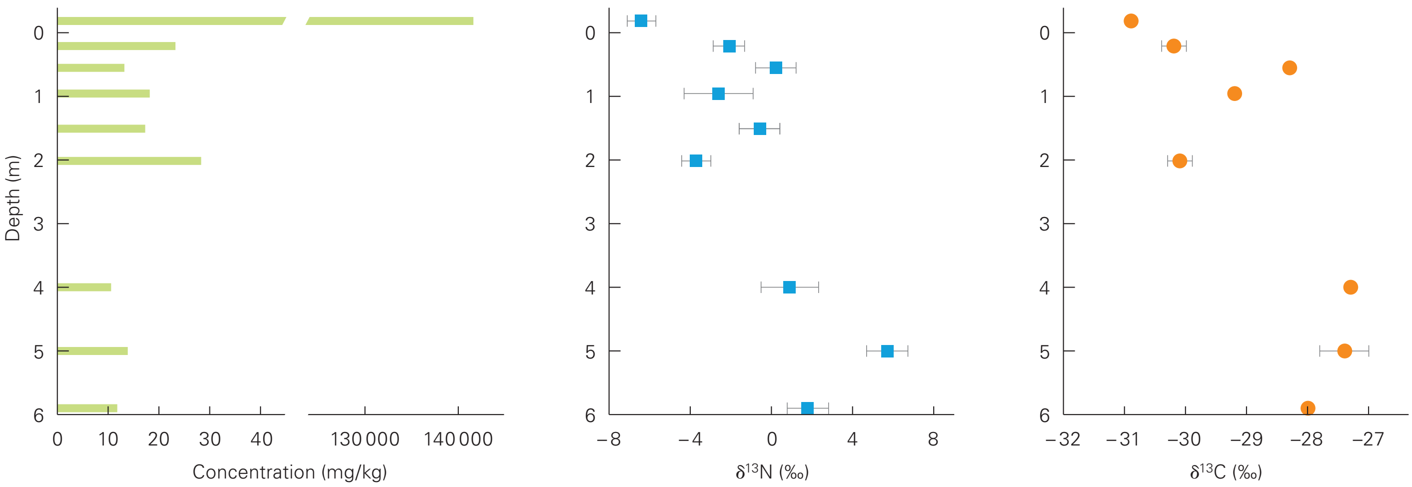


Fig. 2: Concentration profile for the explosive 2,4-DNT over a depth of 6 metres and the carbon and nitrogen isotope signatures (adapted from [7]).

benzotriazoles and methylbenzotriazoles produced by different manufacturers differ mainly in the nitrogen isotope ratios. This can be explained by the routes used for synthesis. While the benzene exhibits $\delta^{13}C$ values between −25 and −30 ‰, which are typical for petrochemicals, the triazole ring has to be produced from chlorinated nitrobenzene in a series of reaction steps. Moreover, other nitrogen-containing compounds such as ammonia and nitrous acid are also used. The widely varying $\delta^{15}N$ values of the benzotriazoles thus reflect the nitrogen isotope ratios of these precursors. In addition, during the synthesis of industrial chemicals, the isotope ratios of the reactants and products are modified because chemical reactions rarely go to completion. The combination of these two factors – the isotope ratios in the precursors and their modification by chemical reactions – produces the benzotriazoles' isotopic fingerprint.

We also investigated whether the benzotriazoles in dishwasher detergents available in Switzerland originate from different chemical manufacturers (Fig. 1c). We found that, with one exception, the nitrogen isotope signatures are very similar (–5 ‰), which suggests that most of the benzotriazoles are produced by the same manufacturer. In future studies, we plan to investigate whether benzotriazoles in Swiss rivers reflect the findings for dishwasher detergents. To this end, a new project involving CSIA of micropollutants has been launched by Eawag, in collaboration with the University of Neuchâtel and

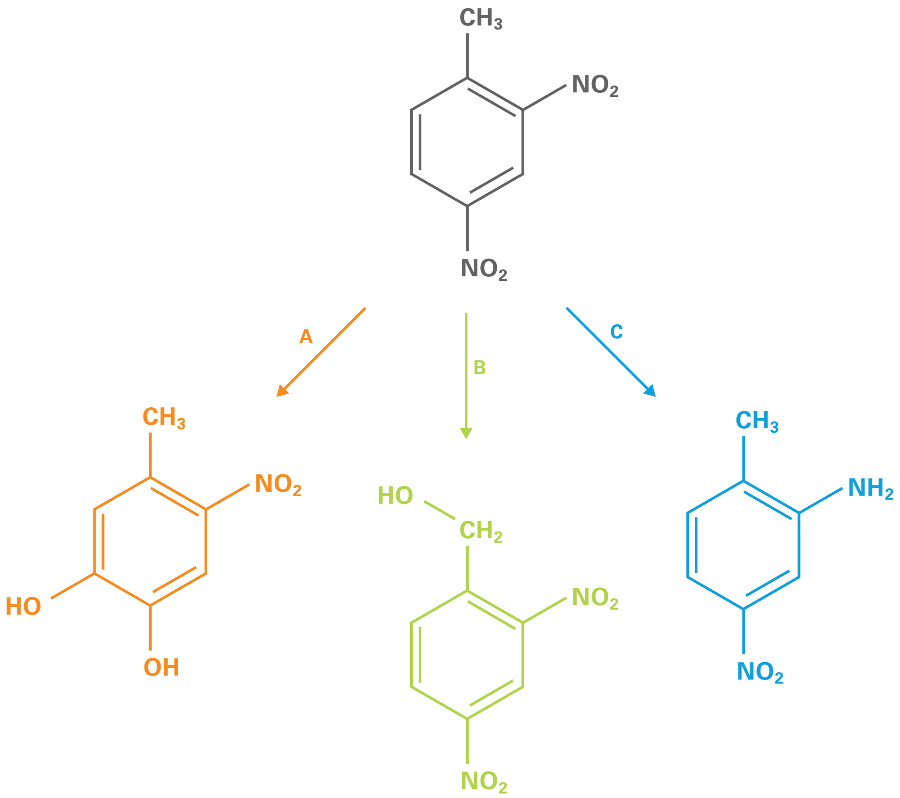


Fig. 3: Initial reaction steps in the microbial biodegradation of 2,4-DNT via dioxygenation (A), methyl-group oxidation (B) and nitro-group reduction (C, only one of two possible products shown). For 2,4-DNT, only dioxygenation leads to mineralization.

the German Research Center for Environmental Health (Helmholtz Zentrum München), with support from the Swiss National Science Foundation.

Reconstructing the degradation of explosives
Nitroaromatic compounds such as the explosive trinitrotoluene (TNT) and its precursor dinitrotoluene (DNT) occur as toxic substances at sites contaminated with explosives or in the polluted subsurface of former production sites. Although their degradation pathways are known, it is extremely difficult to estimate the extent and rate of degradation of this class of substances in the environment. This is because degradation takes place over decades. Secondly, isotopic analysis is complicated by the fact that both the substances and their transformation products are often strongly bound to the organic and mineral matrix of soils or sediments. In addition, many nitroaromatic compounds are transformed via a number of reactions that may give rise to even more toxic products, such as aromatic amines.

At a contaminated site in Switzerland, Eawag has now, for the first time, successfully evaluated the biodegradation of nitroaromatic compounds on the basis of changes in isotope signatures – so-called isotope fractionation [7]. Figure 2 shows a concentration/depth profile for 2,4-DNT at the highly contaminated spot. Comparable data are available for TNT and 2,6-DNT. The concentrations measured in the subsurface indicate transport of the contaminants to the subsurface. Whether degradation also occurs in this process cannot be determined from these data alone. It is known, however, that microorganisms can degrade 2,4-DNT via three different reactions, only one of which leads to mineralization (Fig. 3).

The changes observed in the C and N isotope signatures of 2,4-DNT with increasing depth provide information on microbial degradation. The lower the concentrations of DNT in the subsurface, the more strongly do the $\delta^{13}C$ and $\delta^{15}N$ values differ from soil-surface samples. This observation provides robust evidence of (bio)chemical transformation. Substantial isotope fractionations of this kind cannot be caused by transport processes and require the cleavage of chemical bonds in processes such as microbial degradation. This physicochemical phenomenon is known as a kinetic isotope effect. As kinetic isotope effects vary depending on the element and the type of chemical bonds broken, isotope fractionation can also be used to identify the reaction mechanism and thus the degradation pathway in the environment [8].

When $\delta^{13}C$ and $\delta^{15}N$ values from Figure 2 are plotted against each other, a systematic trend towards heavier carbon and nitrogen isotope signatures can be seen for 2,4-DNT. With the aid of typical isotope fractionation trends observed for 2,4-DNT degradation in the laboratory, this trend can be assigned to specific degradation pathways (Fig. 4). If a contaminant is simultaneously eliminated by different mechanisms, the isotope fractionation trends reflect a combination of the various degradation processes. Accordingly, the isotope signatures observed in the contaminated subsurface show that 2,4-DNT was largely eliminated by dioxygenation, which leads to complete mineralization.

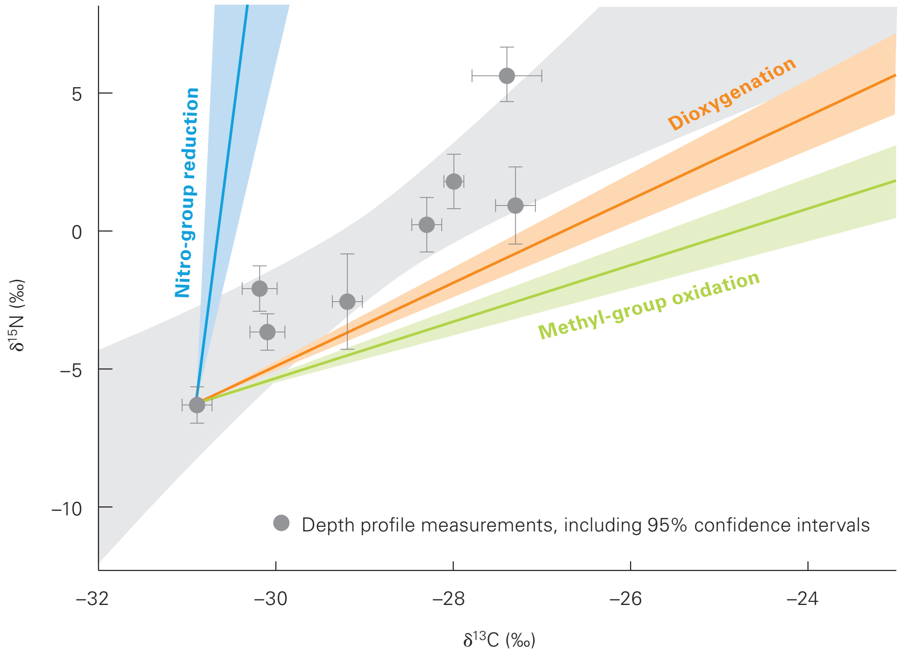


Fig. 4: Isotope fractionation analysis of 2,4-DNT in a contaminated subsurface (grey circles). The enrichment of ^{13}C and ^{15}N in the course of degradation follows trends observed in laboratory experiments for mineralization via dioxygenation (orange trend curve) and for reduction to aminonitrotoluenes (blue trend curve) (adapted from [7]).

The remainder was transformed by nitro-group reduction to problematic aminonitrotoluenes. Compounds of this kind were also detected in our samples.

In order to use the instrument of CSIA in a more quantitative way, we are currently investigating the extent of isotope fractionation and the underlying kinetic isotope effects of various reaction mechanisms responsible for the biodegradation of nitroaromatic compounds. Initial laboratory studies with 2,4-DNT show that, for a given quantity of substance transformed, dioxygenation causes relatively low fractionation of carbon and nitrogen isotopes. As this is the predominant degradation reaction, the high isotope fractionation observed at the field site indicates that a large proportion of this contaminant (over 99 per cent) must have been mineralized. Owing to the high levels of contamination, however, the contaminant was still detectable. If one compares the estimated quantities degraded with historical data for production periods, a half-life of 10–50 years can be calculated for 2,4-DNT in the subsurface. Even if the uncertainty associated with the degradation rate amounts to one or two decades, CSIA provides information on the extent and pathway of contaminant transformation which cannot be obtained by conventional methods.

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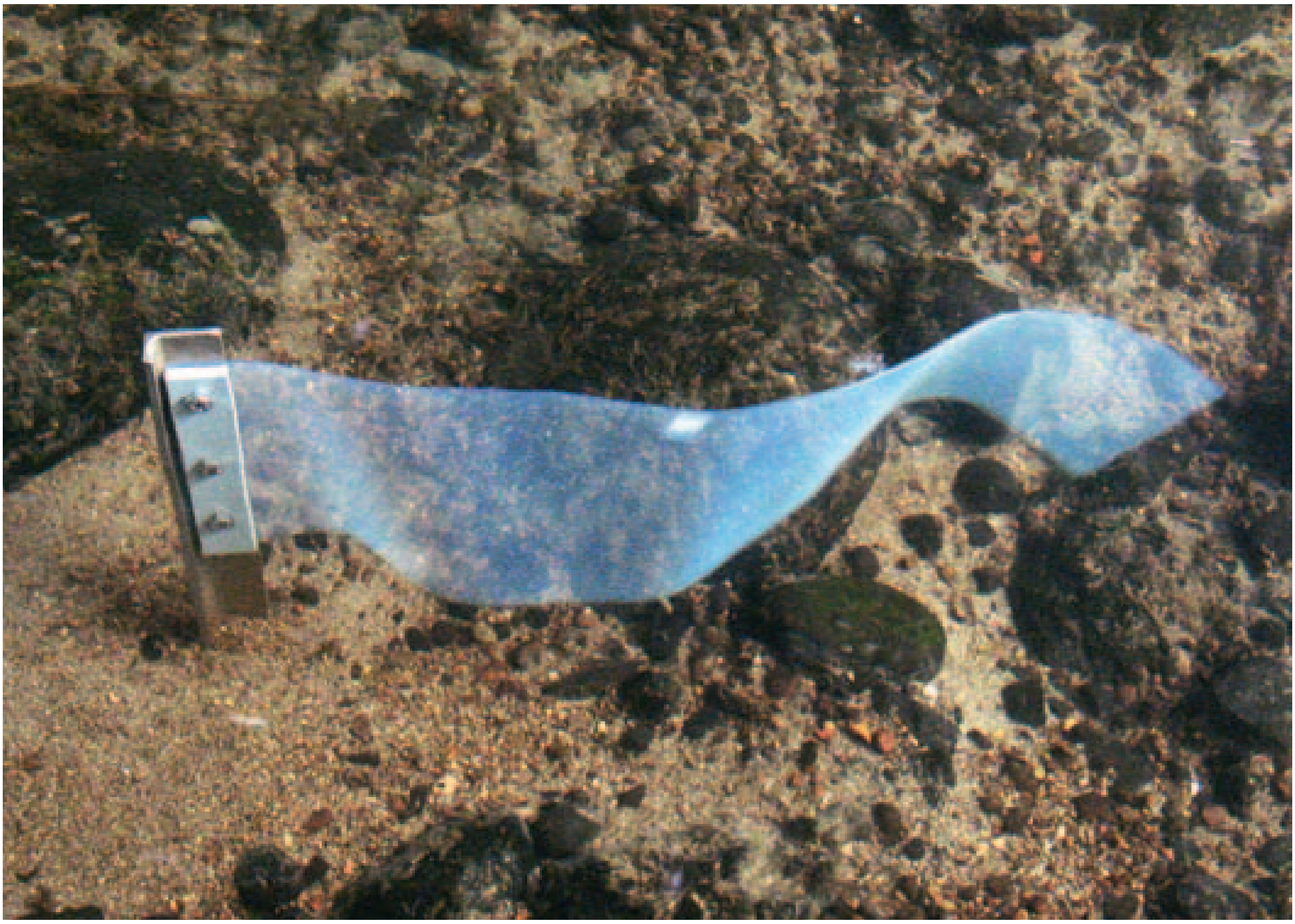
Advantages of passive sampling

With passive sampling, contamination of rivers can be analysed more comprehensively than with spot sampling. The method is suitable for capturing spatial and temporal patterns of pollutant inputs. It can also be used to detect substances present in waters at concentrations below the limit of quantification – for example, PCBs in the Birs river.



Etiënne Vermeirssen

Chemicals in water diffuse into a passive sampler, accumulating in the silicone sheet.

Pollutants enter our surface waters by a wide variety of pathways. Many of these substances can now be reliably measured, and new methods allowing detection of additional substances are constantly being developed. All these analyses are dependent on the collection of water samples, which frequently involves spot (or "grab") sampling. But to what extent does analysis of individual spot samples provide an accurate and representative picture of the pollutant situation?

Inadequacy of spot sampling

Watercourses are highly dynamic systems. As a result of rainfall, flow rates vary continuously, leading to differences in the dilution levels of pollutants. Inputs to surface waters are also dynamic, with run-off of pollutants from roads (often metals) or agricultural areas (often pesticides) occurring intermittently. Inputs from industrial processes also vary significantly over time. In addition, because it takes some time for substances entering a river or stream to be completely mixed with the water, they are not homogeneously distributed. All these factors make it very difficult to collect representative samples. In particular, if one wishes to find out whether water quality standards are being complied with or to determine where action is required, individual measurements are not sufficient for an assessment. Automatic samplers are only suitable for use at certain sites, such as outflow pipes or well-mixed river sections. Operation of such systems is also expensive and labour-intensive, which makes them less suitable for large-scale monitoring programmes.

An alternative is offered by passive sampling, where substances in water are retained by and accumulate in a sorbent material (see Box). This means that compounds can also be detected which are otherwise difficult to determine in a water sample because of low concentrations. Originally developed for the analysis of air pollutants, passive sampling has also been used over the last three decades to detect metals and hydrophobic organic pollutants in water [1, 2]. Methods for sampling hydrophilic organic substances in water were first introduced ten years ago. Researchers at Eawag and the Eawag/EPF Lausanne Ecotox Centre have been studying passive samplers for some time. Below, we discuss two examples illustrating the advantages of the method.

PCB source identified on the Birs

In 2008, the discovery of fish in the Birs river (Canton Jura) with concentrations of dioxin-like polychlorinated biphenyls (PCBs) far above the permissible limit was widely reported [3]. The cantonal authorities advised anglers to restrict their consumption of fish and even imposed a ban on fishing in certain particularly contaminated reaches. However, no indication of the possible source of PCB pollution was found in the fish. Therefore, at the suggestion of the Canton Jura environment agency and on behalf of the Federal Office for the Environment, a team of researchers from Eawag's Environmental Chemistry and Empa's Analytical Chemistry department collaborated with Cantons Bern, Basel-Landschaft and Jura to investigate whether passive sampling could facilitate the search for the source of the contaminant. This method appeared to us to be particularly suitable since passive samplers – like fish – continuously accumulate PCBs. Based on previous experience, we selected the polymer polydimethylsiloxane (silicone) as the sampling phase [2].

Silicone sheets were deployed at ten sites along a roughly 60-kilometre stretch of the Birs. After an exposure period of several weeks, we extracted PCBs from the samplers and established the contamination of the river. Thanks to these analyses, it was possible to pinpoint the source of the inputs (Fig. 1). A second measurement campaign subsequently identified an industrial site as the source of contamination. Remediation measures were then rapidly initiated by Canton Jura so as to prevent further inputs of PCBs. Initial follow-up controls indicate that the measures have been worthwhile, as contamination has decreased sharply. Following the success of this study, silicone passive samplers have also been used for PCB monitoring in the Saane and Aare rivers and in the Rhône catchment.

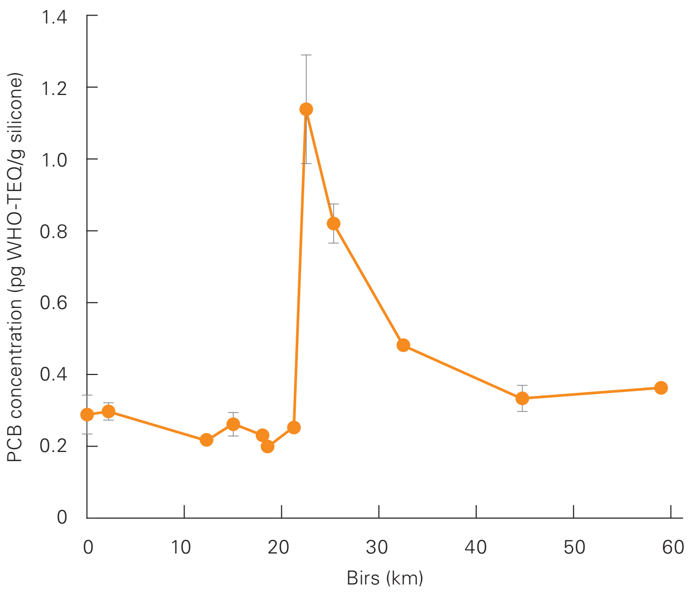


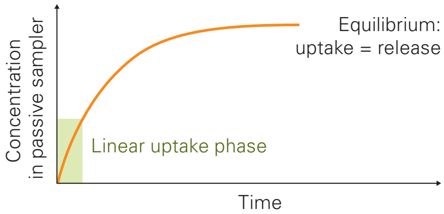
Fig. 1: PCB concentrations (expressed as toxic equivalents) measured along the Birs river. The input source is clearly identifiable.

Passive samplers

In passive sampling, substances diffuse from the sampled medium (e. g. water, known as the donor phase) into the receiving phase of the device. The process is driven by a difference in the chemical activity of substances in the two phases. Compounds are retained on the surface of the sampler, where further diffusion and sorption processes occur. Depending on the target compound, lipids, solvents or polymers may be used as the receiving phase.

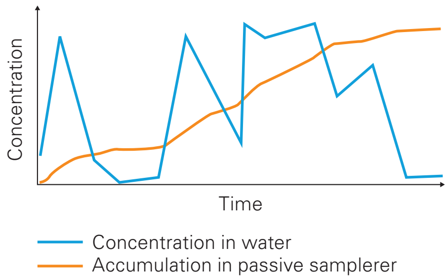
Substance flow from the water phase to the receiving phase is proportional to the difference between the chemical activities in the two phases. If this difference is large enough, a compound will accumulate continuously in the sampler. Initially, accumulation proceeds at a constant rate and the curve is practically linear. For accurate sampling, it is important that the exposure duration should fall within this phase, well before a state of equilibrium is reached (see top figure). Fluctuating concentrations in the water lead to differences in the amounts of a substance that accumulate at different time points. In this way, variable concentrations can be integrated over a given period (see middle figure).

Passive samplers widely used for analysis of organic pollutants are Chemcatcher and POCIS (polar organic chemical integrative sampler). In these devices, a solid receiving phase is covered by a membrane (see photos on the right). The samplers are mounted in holders for deployment in rivers. After an exposure period of several weeks, the devices are retrieved and substances are extracted from the receiving phase and analysed.



As the rate of accumulation of a substance in a passive sampler is constant in the linear phase under stable environmental conditions, the increase in the amount of substance in the sampler is proportional to the concentration of the substance in the water.

Substance uptake at a constant concentration in water. The concentration in the sampler increases until equilibrium is reached between uptake and release.



Various types of passive samplers. a) Silicone sheets for sampling of polychlorinated biphenyls, b) POCIS device, c) Chemcatcher for sampling of hydrophilic organic contaminants.

Regula Haag, Etiënne Vermeirssen

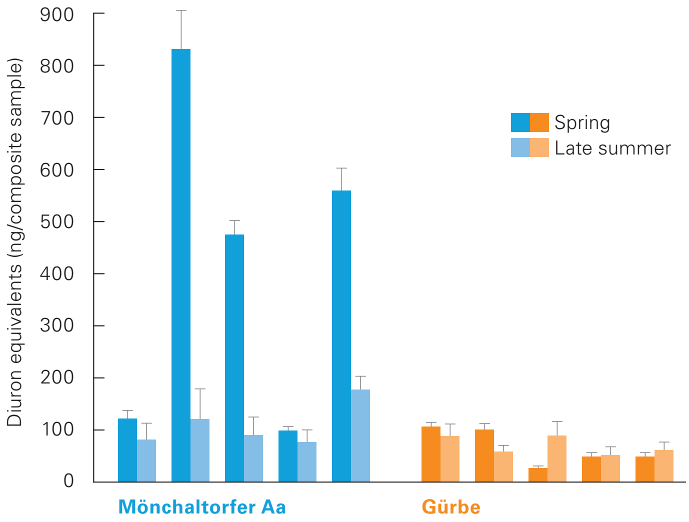
Passive sampling combined with bioassays

Passive sampling was also employed in the "Integrated River Water Quality Management" project, a collaborative effort involving several Eawag research departments (www.eawag.ch/iwaqa). The project – part of the National Research Programme "Sustainable Water Management" (NRP 61) – aims to characterize in detail the state of the Mönchaltorfer Aa and Gürbe river catchments. Decision-making support is to be developed to permit the assessment and minimization of adverse effects on the ecological state of watercourses. The influence of various management measures over the long term is to be assessed with the aid of a mathematical model. On both rivers, passive samplers were deployed in order to obtain more representative average pollutant values than would have been possible with low-frequency spot sampling. In addition, after prolonged exposure, the samples collected were sufficiently large to allow water quality to be assessed by means of various chemical analyses and bioassays.

Samples were collected from both rivers over a two-week period using Chemcatcher devices (see Box). The sampling phase used was a granulated sorbent fixed in a polytetrafluoroethylene (Teflon) matrix. Water quality was then assessed at the Ecotox Centre with the aid of three different bioassays. An algal assay was used to analyse samples for herbicides that inhibit photosynthesis. Using an enzyme inhibition assay, it was possible to detect insecticides that disrupt signal transmission in nerve cells. Lastly, genetically modified yeast cells were used to screen for substances with estrogenic activity.

As is shown by the algal assay, for example, passively collected samples provide a detailed picture of river water quality. Firstly,

Fig. 2: Herbicide concentrations observed at five sampling sites in the Mönchaltorfer Aa and Gürbe rivers. The values given are averages calculated for six passive samplers (composite samples), expressed as "diuron equivalents". The diuron equivalent concentration is the concentration of the herbicide diuron that produces the same biological effects in an algal assay as the herbicide mixture present in the sample.



they effectively revealed spatial variation of pollutant concentrations in the Mönchaltorfer Aa and Gürbe catchments. Thus, herbicide concentrations were found to be markedly higher in various sections of the former river than in the latter. At the same time, passive sampling also revealed temporal variability: for both rivers, samples collected in May showed higher herbicide concentrations than in the late summer (Fig. 2). This reflects the increased application of crop protection products at the start of the growing season. In a previous study, we had already demonstrated that passive sampling is an effective method for the determination of pesticide concentrations in treated sewage effluents [4].

Taking environmental factors into account

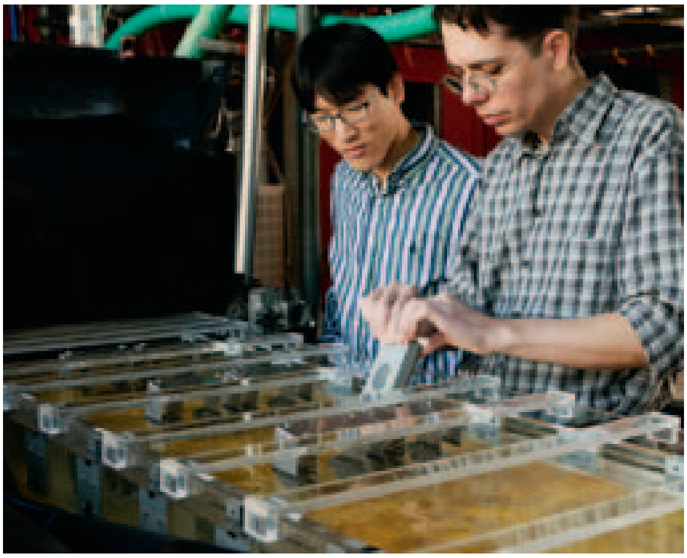
Although passive sampling – in combination with chemical analysis or bioassays – has already produced useful results, there is still room for optimization. For example, not all substances accumulate in a sampling phase at the same rate. Compared to mixtures of substances that accumulate slowly, the toxicity of rapidly accumulating mixtures will tend to be underestimated in passive sampler extracts, even if both mixtures would be equally toxic to aquatic organisms.

The diffusion processes which determine the uptake of chemicals depend on environmental factors, such as temperature or flow conditions, and on the dimensions and design of the sampler. The question of how flow rates and diffusion-limiting membranes affect uptake in passive samplers has been studied by the Environmental Chemistry and Environmental Toxicology departments of Eawag. For this purpose, we set up a modular channel system for river or wastewater, which can be spiked with chemicals (Fig. 3). It was shown that in passive samplers without membranes (e.g. silicone sheets or Chemcatcher devices with naked disks) the rate of accumulation is higher at a higher flow rate. This is because, with increasing flow rates, diffusion paths become shorter as a result of hydrodynamic effects. Such findings can be used to normalize field data and improve assessments.

Difficulties in determining concentrations

If passive sampling data (amount of substance per sampler per unit time) are to be used to determine substance concentrations in water, the samplers need to be calibrated. Calibration may be carried out in the laboratory. This process involves exposing passive samplers for different periods to water spiked with chemicals and determining uptake kinetics on the basis of the amounts accumulated. Alternatively, field calibration is also possible. Here, samples are collected at regular intervals in an initial measurement campaign. The sampling rate can then be calculated from the average substance concentration in the water, the amount taken up by the sampler and the exposure period. For example, if the average concentration is 5 nanograms per litre and 50 nanograms is taken up over a 10-day period, then the sampling rate is 1 litre per day. In subsequent measurements, the amount of substance accumulated is divided by the sampling rate and exposure time to obtain the estimated substance concentration in the sampled water [5].

This approach works well if conditions in the field are similar to those under which calibration is performed. But in reality,



Stefan Kubli

Fig. 3: In a modular flow channel experiment, Junho Jeon and Etiënne Vermeirssen investigate how the sampling rate in passive samplers is affected by the membrane and environmental conditions [6].

environmental conditions, such as flow rates, vary continuously. So, even if sampling rates are known, the determination of substance concentrations by means of passive sampling also involves uncertainties. When bioassays are used, it is often not possible to estimate concentrations, as in such cases the samples analysed are usually mixtures. If it is not known what substances are present in the mixture, sampling rates cannot be determined and the toxicity of a sample can only be expressed in terms of amount per sampler rather than per litre of water.

Need for optimization and standardization

To improve the reliability of information on substance concentrations provided by passive sampling, further research is required [5]. But despite the limitations in this area, the method is already suitable for obtaining spatially resolved data on pollutant concentrations in rivers and identifying sources of contamination. In addition, temporal patterns of inputs can be reliably determined and followed over extended periods. Passive sampling delivers considerably more meaningful results than individual grab samples and is inexpensive and not unduly labour-intensive.

Another advantage lies in the fact that, over time, larger quantities of substances accumulate in passive samplers. Accordingly, it may be possible to detect substances – as in the case of PCBs in the Birs – because concentrations in the sampler are above the limit of quantification, or – as in the project on the Mönchaltorfer Aa and Gürbe rivers – to analyse samples using several different methods. Within the international NORMAN network of reference laboratories, to which Eawag and the Ecotox Centre belong, efforts including large-scale interlaboratory tests are currently under way to further optimize and standardize passive sampling.

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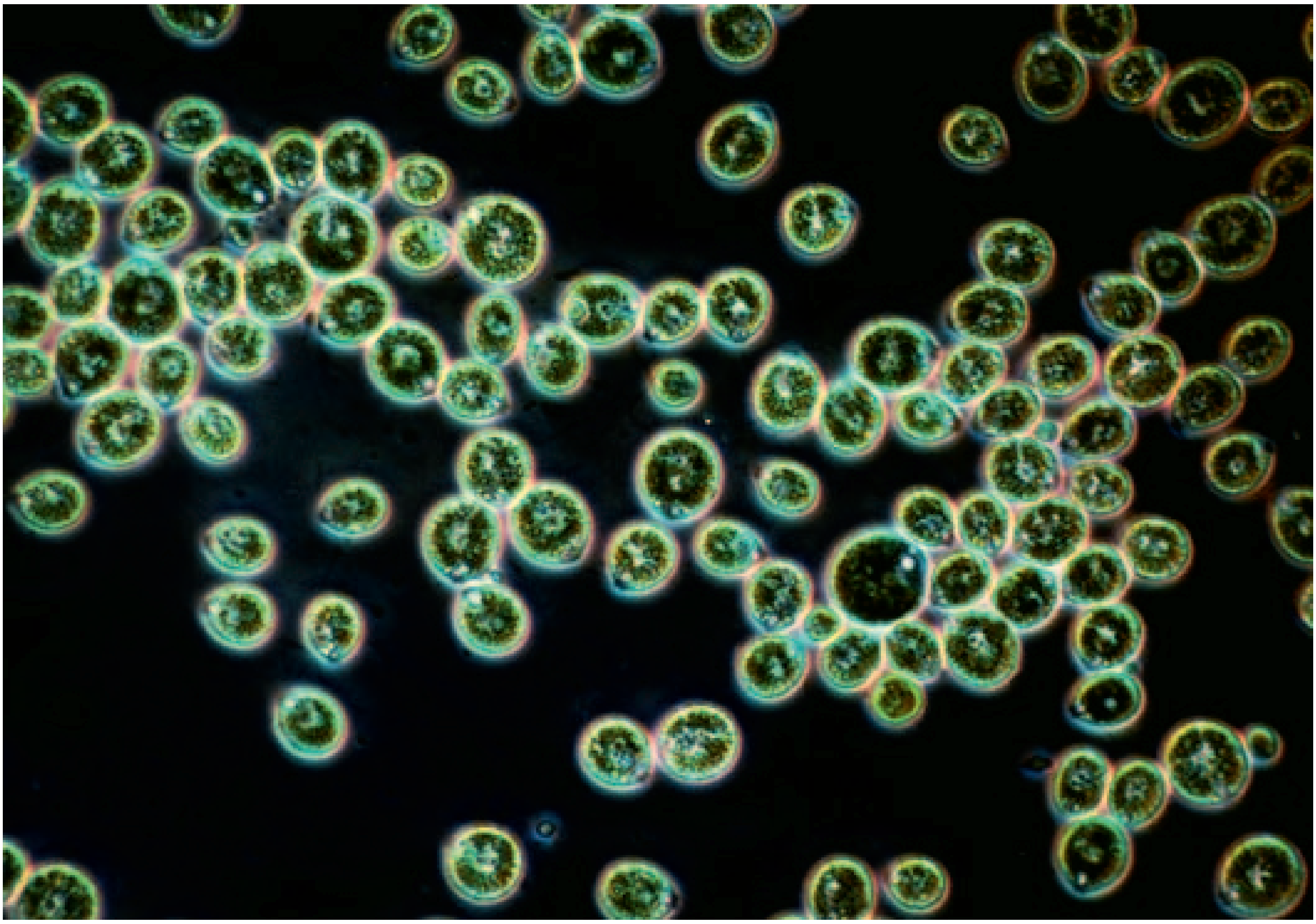
Smitha Pillai, ecotoxicologist, studies the effects of environmental chemicals on genes and proteins.

Regulation of cellular functions altered by silver

Traditional methods of assessing the effects of environmental toxicants are often inadequate – especially if these substances occur at low concentrations. But with the so-called omics methods, effects can be detected at the molecular level – for example, alterations of gene expression and protein profiles in green algae exposed to silver.



Okapia

Silver ions disrupt cellular metabolism in the green alga *Chlamydomonas reinhardtii*, inhibiting functions such as photosynthesis.

In 1997, approximately 13,420 tonnes of silver entered the environment worldwide, including around 400 tonnes released directly to surface waters in industrial and municipal wastewater [1]. This toxic heavy metal is persistent and can bioaccumulate in the food chain [2]. For some years now, a major source of silver in the aquatic environment has been nanoparticles, which – on account of their antibacterial properties – are widely used in textiles, detergents, cosmetics, outdoor paints and even in agricultural products [3]. In Switzerland, concentrations of silver nanoparticles in surface waters are now estimated at 30–80 nanograms per litre [4]. Various studies have shown, however, that toxicity is attributable not to silver nanoparticles

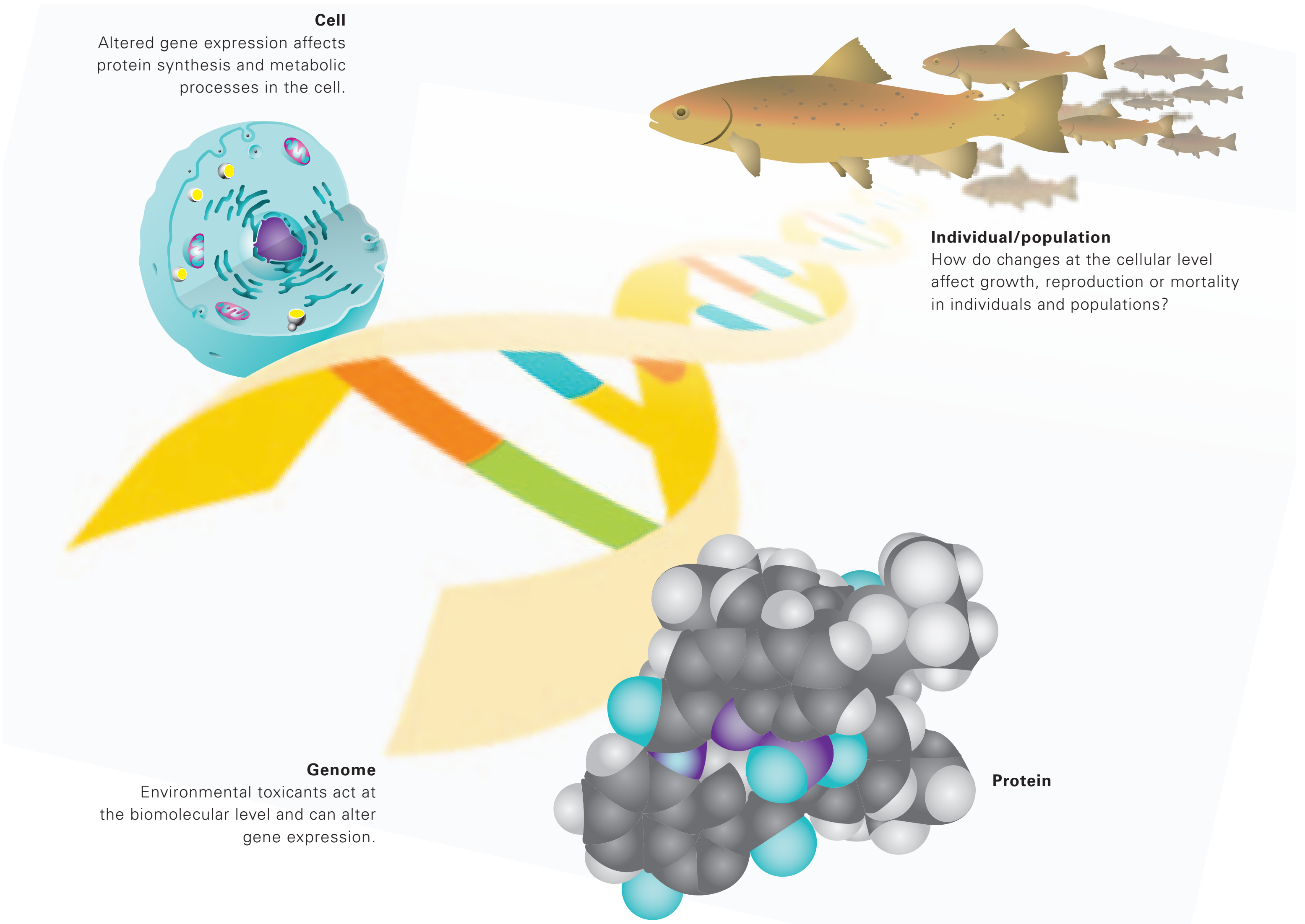


Fig. 1: The effects of environmental toxicants need to be studied at different levels of biological organization.

themselves, but to the free silver ions released by them [5], which pose a serious risk to aquatic organisms.

Concentrations of ionic silver in natural waters are generally low – up to a few nanograms per litre – because silver ions can complex with sulphides and chlorides, adsorb on sediment and precipitate. Nonetheless, even at nanomolar concentrations, free silver ions are toxic to a variety of aquatic organisms. For example, algae are particularly at risk owing to the high potential for bioaccumulation of dissolved silver. As algae are a food source for many organisms, this contaminant can accumulate along the food chain, with higher-level organisms also being exposed to problematic concentrations.

Bridging different levels of biological organization

Assessing the environmental risk of a toxicant usually involves carrying out standardized laboratory experiments to determine how it affects growth, reproduction or survival in model organisms (e.g. yeast cells, green algae or water fleas). The findings are then used to establish guidelines for the management of toxic substances in the environment [6]. However, this approach has a limited ability to detect effects of toxicants at low concentrations. In such cases, deleterious effects may not be observed in traditionally endpoints such as reproduction or survival, but more subtly at the subcellular or molecular level. Accordingly, it is essential to assess the effects of substances such as silver across various levels of biological organization – from the molecular, cellular and organism level to whole populations and ecosystems (Fig. 1).

In organisms exposed to chemicals, all primary interactions take place at the level of the biomolecules of which the cells are composed. To determine how environmental toxicants affect an organism, it is therefore vital to understand the underlying molecular mechanisms. This should also permit a more detailed ecotoxicological risk assessment. Thanks to recent advances in biological, analytical and bioinformatic methods, researchers now have at their disposal suitable technologies for investigating effects at the molecular level. With a systems biology approach, processes can be studied globally using the "omics" methods: *genomics* simultaneously analyses every gene present in an organism (genome), while *transcriptomics* analyses gene expression, i. e. all the RNA molecules produced according to the genetic instructions in the DNA (transcriptome). Likewise, *proteomics* studies the complete set of proteins (proteome), and *metabolomics* the small-molecule metabolites (metabolome).

Measuring molecular responses

Although the genome is essentially the same in every cell of an organism, different cells show different patterns of gene activity, or expression, to serve various physiological functions – and also in response to external stimuli, such as chemical stressors. "Gene expression" refers to the process in which RNA is synthesized, or transcribed, from a gene; thus, the transcriptome constitutes a snapshot of all actively expressed genes at a given time and under given conditions [7]. Using DNA microarrays and next-generation sequencing methods, it is possible to analyse how the transcriptome responds when an organism is exposed to environmental toxicants (see Box). From the specific patterns of gene expression induced, conclusions can be drawn about a substance's mode of action.

RNA transcripts, in turn, serve as the blueprint for the synthesis of proteins (translation). Accordingly, analysis of an organism's protein profiles (proteome) offers additional insights into the biological pathways that may be regulated upon exposure to a toxicant. The proteome can be analysed with the aid of liquid chromatography coupled to mass spectrometry (see the diagram below), which is also used to assess the metabolome. The metabolome provides a more realistic assessment of the physiological state of an organism than the transcriptome or proteome. If the omics technologies are used in combination with traditional ecotoxicological methods, molecular and biochemical reactions in cells can be causally described and quantitatively analysed [8]. This information when subsequently fed into population-level modelling efforts can be used to assess ecotoxicological risks, on the basis of sound mechanistic data.

Altered gene expression

At Eawag, omics methods have been used, for example, to study the toxicity of silver in *Chlamydomonas reinhardtii*. This unicellular, photosynthetic green alga is an important model organism for ecotoxicology, representing the phytoplankton,

Transcriptomics

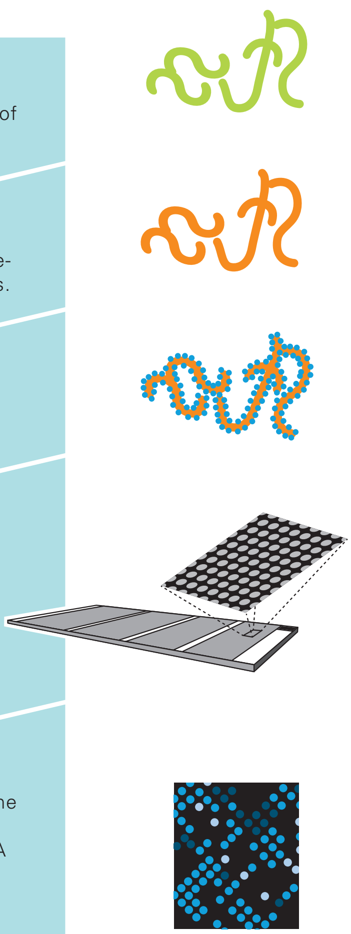
Isolation
All the RNA (including transcriptome) is isolated from the cells of an organism.

Complementary RNA
The RNA is converted to complementary RNA (cRNA) sequences.

Labelling
The cRNA is labelled with the fluorescent dye Cy3.

Hybridization
Labelled cRNA is applied to microarrays which contain DNA sequences representing the organism's entire genome. The cRNA binds to the complementary DNA sequences.

Detection
Based on fluorescence levels, the gene expression profile can be determined, and expressed DNA sequences can be assigned to specific genes and functions ("annotation").



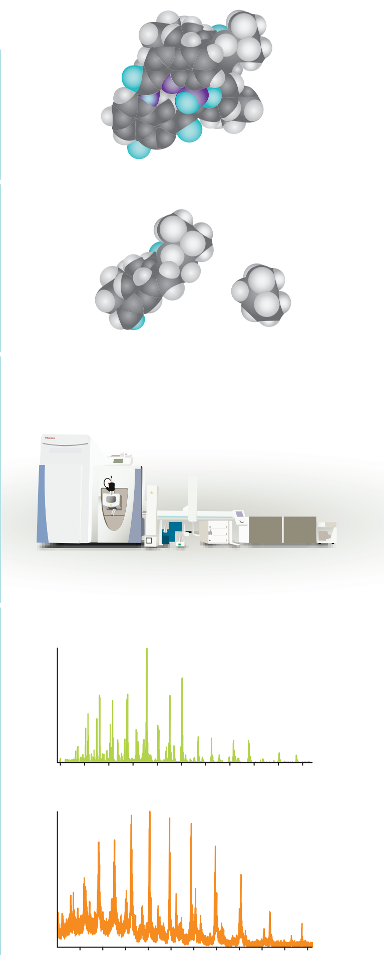
Proteomics

Extraction
All the proteins (proteome) are isolated from the cells of an organism.

Digestion
With the enzyme trypsin, the proteins are broken down into their component peptides.

Mass determination
Using multidimensional protein identification technology (MudPIT), the masses of individual peptides are determined in a mass spectrometer.

Identification
The mass spectra of individual peptides are used to identify and quantify the proteins present in the cells.



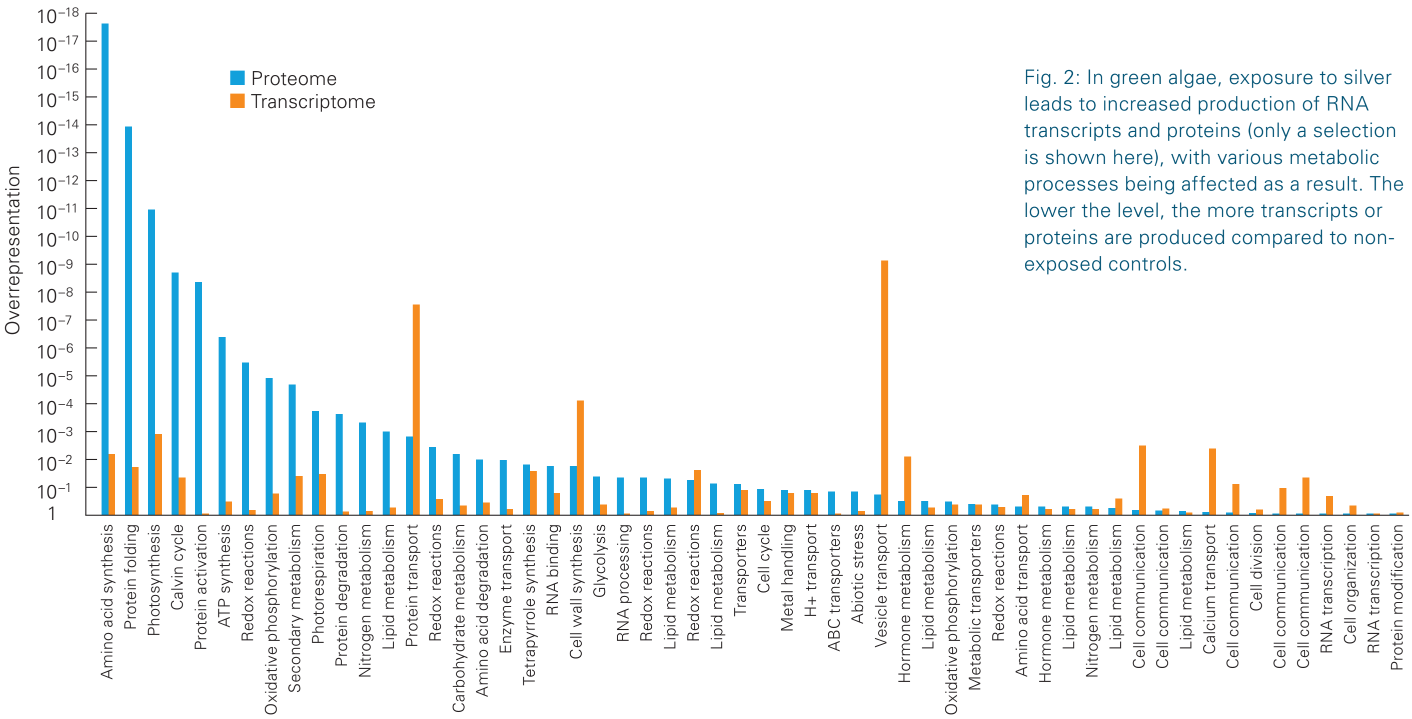


Fig. 2: In green algae, exposure to silver leads to increased production of RNA transcripts and proteins (only a selection is shown here), with various metabolic processes being affected as a result. The lower the level, the more transcripts or proteins are produced compared to non-exposed controls.

which forms the base of the aquatic food chain. *C. reinhardtii* can be cultivated easily in laboratory conditions and, most importantly, its genome is completely sequenced. Dissolved ionic silver is rapidly taken up by *C. reinhardtii* and bioaccumulates in the cells. It is toxic in nanomolar concentrations and has adverse effects on several important metabolic pathways; for example, it inhibits growth and photosynthesis. Although these effects can also be demonstrated with traditional exposure experiments, the underlying mechanism of action remains unclear. We are therefore studying the changes which can be observed in the transcriptome, proteome and metabolome of *C. reinhardtii* in response to silver.

In initial experiments, the green algae were exposed to varying amounts of silver, and the transcriptome and proteome responses were analysed using microarrays and MudPIT approaches, respectively. In the exposed algae, several thousand transcripts were significantly regulated as compared to the non-exposed controls – i. e. expression of the genes concerned was affected by exposure to silver. Similarly, at the proteome level, nearly a thousand proteins were differentially regulated. Most of the transcripts and proteins could be correlated with each other and functionally categorized (Fig. 2). Changes in the transcriptome and the proteome were observed at environmentally relevant concentrations, although they were more marked at higher concentrations.

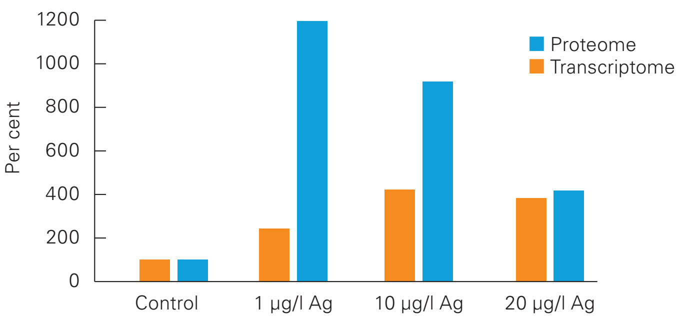


Fig. 3: Green algae exposed to silver produce more glutathione S-transferase, a protein which helps to combat oxidative stress. RNA and protein synthesis are increased compared to controls.

Effects on metabolic pathways

The metabolic pathways affected by exposure to silver included those of photosynthesis, pigment production, energy and lipid metabolism, and sugar and starch synthesis. Changes occurring at the transcriptome and proteome level were linked to the physiological state of the cell. In the case of photosynthesis, an imbalance in the electron transport chain leads to the synthesis of reactive oxygen species (ROS). High levels of ROS synthesis produce oxidative stress and can cause oxidation of lipid, proteins and DNA. The cell normally counteracts oxidative stress by a defence response which includes proteins that can detoxify the ROS. In algae exposed to silver, several of these defence response proteins were indeed induced, suggesting that silver causes oxidative stress (Fig. 3). In addition, photosynthetic efficiency and the content of ATP (the primary energy carrier in cells) decreased as silver concentrations increased.

Another good example is the synthesis of lipid bodies – cell organelles which store triacylglycerol (TAG), a precursor of fatty acids. Literature data suggest that synthesis of TAG is triggered when cells are exposed to stress factors, and this was confirmed by our investigations. The number of lipid bodies in algal

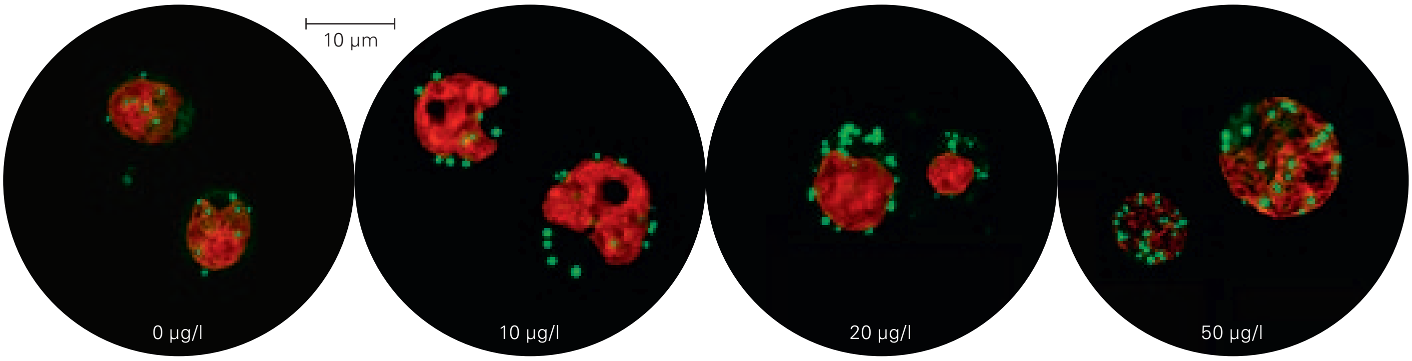


Fig. 4: As silver concentrations increase, more lipid bodies (green) can be seen in the algal cells.

cells increased with increasing exposure to silver (Fig. 4), correlating well with other responses which indicate the induction of oxidative stress. In addition, ionic silver not only affects RNA transcription and protein synthesis, but also interacts directly with proteins. Silver can inactivate proteins, especially if their active sites contain copper. This in turn can cause signals for regulations at the transcriptome and proteome level.

Deducing the mechanism of action

The mechanism of action of silver in *Chlamydomonas* algae can be deduced from analysis of the transcriptome and proteome data and the physiological findings. Ionic silver is actively taken up by the cells and interacts with proteins in several metabolic pathways, thus inhibiting functions such as photosynthesis and ATP production. This triggers oxidative stress, causing damage to proteins and DNA. These effects at the cellular level ultimately lead to adverse effects – e.g. on growth – at the individual and population level.

In cells exposed to environmental toxicants, the omics methods represent a promising approach for elucidating mechanisms of action and thus assessing effects on metabolic processes. Moreover, in addition to conventional parameters such as growth or mortality, new, possibly more sensitive endpoints can be established for the quantification of biological effects. In the case of *Chlamydomonas*, an endpoint of this kind would be the accumulation of lipid bodies. The new technologies are thus of fundamental importance in the development of knowledge-based ecotoxicological risk assessment.

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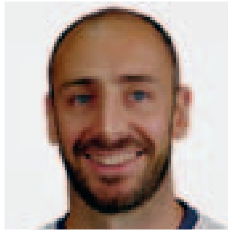


Francesco Pomati, microbiologist, leads a group studying phytoplankton biodiversity in the Aquatic Ecology department.
Co-author: Andri Bryner

An automated approach to lake monitoring

Algae, like other life forms, are subject to biorhythms: the abundance and diversity of phytoplankton vary from daytime to nighttime, as well as from season to season and over the depth of the water column. In research supported by the Swiss National Science Foundation, Eawag and the ETH Zurich have deployed a floating monitoring platform which – for the first time – is providing the high-resolution data that are needed to improve our understanding of the drivers of phytoplankton dynamics.

Freshwater ecosystems typically have a high level of biodiversity. At the same time, they are among the world's most threatened habitats, and extinction rates among aquatic species are escalating [1].

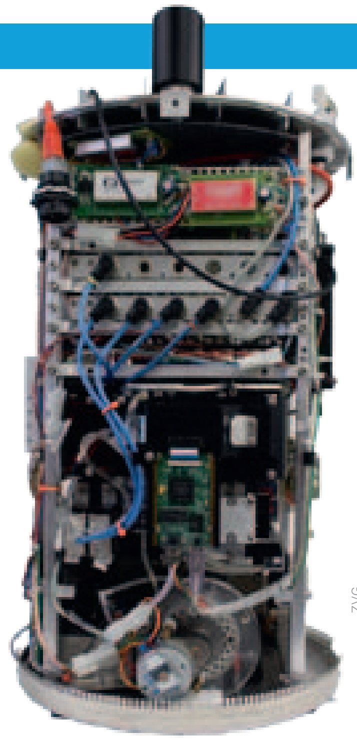
Phytoplankton: the basis of the food web
Phytoplankton – communities of free-floating microscopic plants and bacteria – are at the base of the food web in lake ecosystems; changes in their composition can affect all other lake organisms. However, it remains unclear whether such communities are simply chance assemblies of local species, or whether their distribution and composition are controlled by predictable mechanisms. It is known that changes can occur very rapidly. New conditions can arise within a matter of hours as a result of changes in the weather and lead to unexpected algal blooms (e.g. toxic cyanobacteria). Close monitoring of the spatiotemporal dynamics of plankton and their growth environment is thus of fundamental importance for research – but high-resolution data are lacking.

Typical plankton monitoring programmes are based on monthly – or at best weekly – samples, usually only collected from one lake site and depth. "If you compare that to forest monitoring," says project collaborator Bas Ibelings (Institute F.-A. Forel, Geneva), "it would be like having a look once every few thousand years and then trying to deduce what has happened in the

Dani Steiner

The floating limnological laboratory on Lake Greifen.

meantime." Pristine waters which could serve as a reference have all but disappeared. The task of understanding – let alone managing – aquatic ecosystems is further complicated by the fact that they are exposed to a wide variety of stressors, which may also interact. Harmful algal blooms, for example, can be promoted by climate change, eutrophication and pollution.



The Cytobuoy flow cytometer determines 54 parameters for plankton particles.

ZVG

Floating limnological laboratory
With support from the Swiss National Science Foundation, Eawag has now developed and successfully tested an integrated platform which

► provides automated high-frequency measurements of phytoplankton at different lake depths,

► couples biological monitoring with data about the physical environment, and

► can stream real-time data for modelling and forecasting of phytoplankton dynamics. Data from the monitoring platform are transmitted via a mobile phone network for analysis onshore [2].

At the heart of the integrated monitoring platform is a specially adapted scanning flow cytometer – the Cytobuoy (manufactured by Cytobuoy, Netherlands). This instrument can analyse plankton with a diameter of 1–700 micrometres and up to a few millimetres in length – covering, for example, not only minute picoplankton but also larger, colony-forming cyanobacteria. The particles are scanned by two lasers with different wavelengths and with light scattered at two angles providing information on size and shape. In addition, fluorescence emitted by photosynthetic pigments is detected as signals in different wavelength ranges, indicating chlorophyll a, phycocyanin and phycoerythrin, or degraded pigments. Based on a total of 54 particle descriptors, each plankton particle can be assigned to a morpho-functional group. While this does not precisely reflect taxonomic diversity as determined by laborious cell counting under the microscope, it does provide a sensitive indication of plankton diversity, with solid reproducibility. It is well known that the quality of microscopic counts of phytoplankton is highly dependent on the skills of the individual operator, and repeatability is often poor as a result. In contrast, repeated analysis of the same sample using our new, automated method produces consistent findings that effectively describe the diversity of dynamic natural phytoplankton communities.

Combination with a multiparameter probe
A pressure sensor on an Ocean Seven (O7) multiparameter probe (Idronaut, Italy) ensures that samples for the flow cyt-

Zurich Cantonal Laboratory, René Schittli

Mass growth of cyanobacteria in Lake Greifen in August 2011.

More frequent restrictions on bathing?

Unlike the EU and other countries (e. g. Australia), Switzerland does not have bathing water quality requirements for algal toxins. In the summer of 2011, when mass growth of the cyanobacterium *Microcystis aeruginosa* occurred in Lake Greifen, experts from Eawag and Zurich University provided support for the authorities (Cantonal Laboratory and Office for Waste, Water, Energy and Air) in assessing the situation.

Fortunately, the algal strain responsible for the blooms did not produce toxic microcystins. However, cell densities in excess of 100,000 cells per millilitre were observed in lake water. Even in the absence of microcystins, exposure to these concentrations can lead to allergic reactions or diarrhoeal disorders – for example, in children or people with sensitive skin, or in dogs drinking lake water. Thanks to effective water protection measures, algal blooms have become less common in Swiss lakes since the 1970s. Whether they are now increasing again, as a result of climate warming, is one of the questions which the monitoring platform can help to address [4]. The situation could become particularly problematic if substantially more toxic cyanobacteria were to emerge than have been observed to date in Switzerland – for example, *Cylindrospermopsis raciborskii* [5], a species already widespread in Eastern Europe. This underlines the need to intensify online monitoring efforts.

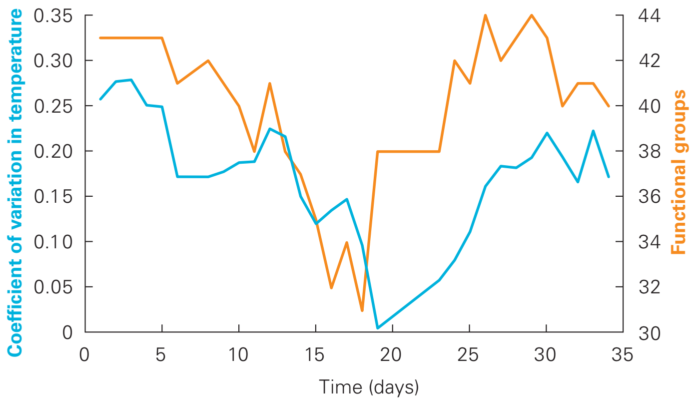


Fig. 2: Phytoplankton diversity (orange curve) in Lake Lugano during the monitoring period (end of April to end of May 2010). The blue curve shows the coefficient of variation in temperature over the water column (0–12 m sampled depth): the higher the value, the greater the thermal stratification. A CV of 0 indicates complete mixing (homogeneous water column temperature).

ometer are collected at defined water depths. However, as well as controlling the sampling tube, the O7 employs additional sensors to determine water temperature, conductivity, pH, and oxygen and nitrate concentrations. Also attached to the probe is a Trilux fluorimeter, which is used to quantify levels of chlorophyll a, phycocyanin and phycoerythrin making it possible to cross-check the results produced by the flow cytometer.

The monitoring platform project is being conducted jointly by Eawag and the Institute of Integrative Biology (ETH Zurich). To ensure that plankton cells are not damaged as water samples are retrieved from the lake, special measures were devised in collaboration with the Eawag workshop: firstly, tubing with an antimicrobial coating is used; secondly, the pump is placed downstream of the plexiglas chamber from which samples are withdrawn for the flow cytometer.

Variation over day-night cycle

In the summer of 2010, the platform was tested for the first time in Lake Lugano – at a site protected from winds and currents – between Laveno and Figino. As shown in Figure 1, the distribution of phytoplankton was found to vary over the day-night cycle – a phenomenon never previously observed in such detail in freshwaters.

As no marked differences were observed in physical parameters (e. g. water temperature) between day and night, it may be assumed that day-night plankton dynamics is driven by biological factors rather than by environmental changes. This theory is supported by the fact that the variation shows a consistent temporal pattern, as is evident from the high-resolution data also obtained for other lakes (e. g. Lake Greifen). The plankton appears to be governed by an "internal clock", and these biorhythms deserve further investigation.

Rapid recovery after mixing

While Lake Lugano was strongly stratified at the beginning of the study period, it became increasingly mixed within about two weeks as a result of falling temperatures and a storm. This development is closely reflected by the plankton data from the monitoring platform: the number of functional groups was initially high and then steadily decreased up to the time at which

From modelling to forecasting

What environmental factors influence plankton community dynamics [3]? The large amounts of highly resolved plankton data generated by the automated monitoring platform facilitate the development of computer models to address this question, and perhaps also improve our forecasting capabilities. We have developed a program based on a multiple linear regression model. The key input parameters are atmospheric and water temperature, conductivity and the heterogeneity of environmental conditions over the water column (coefficient of variation). With these drivers, the model can already account very well for the plankton data collected by the platform. It should be noted that there is generally a time lag of 24 or 48 hours between changes in environmental factors and the response at the plankton community level.

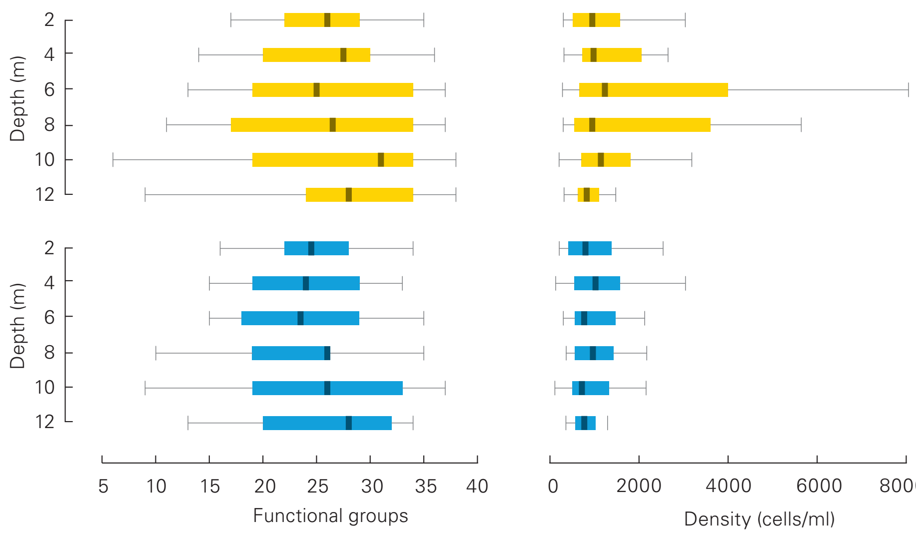


Fig. 1: Both the diversity (number of functional groups) and the depth distribution of phytoplankton vary markedly over the day-night cycle (yellow: 15:00; blue: 03:00).

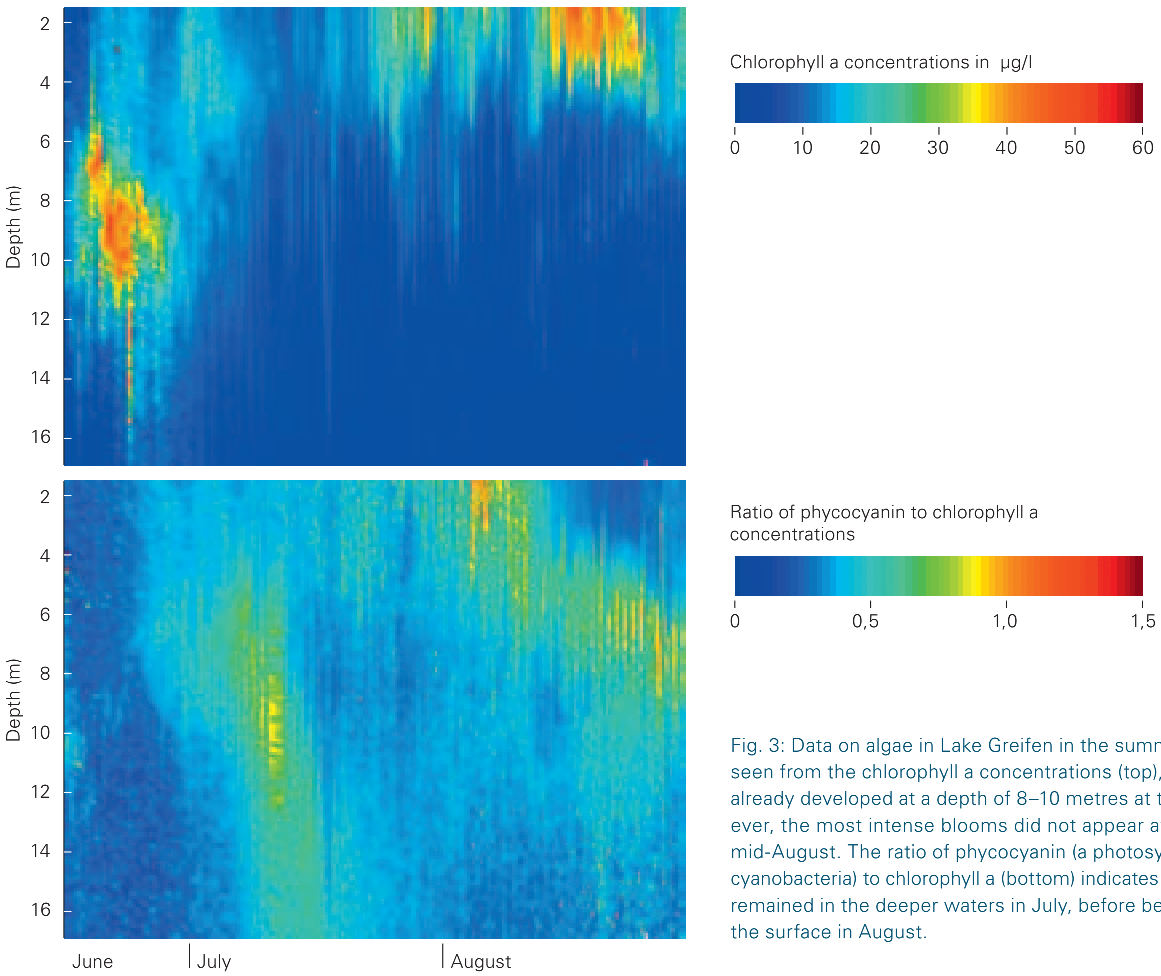


Fig. 3: Data on algae in Lake Greifen in the summer of 2011. As can be seen from the chlorophyll a concentrations (top), large amounts of algae already developed at a depth of 8–10 metres at the end of June. However, the most intense blooms did not appear at the lake surface until mid-August. The ratio of phycocyanin (a photosynthetic pigment in cyanobacteria) to chlorophyll a (bottom) indicates that the cyanobacteria remained in the deeper waters in July, before becoming dominant at the surface in August.

complete mixing occurred (coefficient of variation in temperature = 0). Interestingly, diversity subsequently recovered rapidly, within only a few days (Fig. 2). The mechanisms underlying the dynamics of phytoplankton diversity remain to be elucidated.

Algal blooms in Lake Greifen

Since the early summer of 2011, the monitoring platform has been moored (with the approval of the cantonal authorities) in the northern part of Lake Greifen. Although long data series, held by Eawag and Canton Zurich, are already available for this lake, data from the floating platform should help to further improve our understanding of the lake system. One event in particular – though not appreciated by swimmers – proved very welcome for researchers: during the first monitoring campaign, algal blooms appeared in Lake Greifen (see Box "More restrictions on swimming?"). The immense volumes of data collected have yet to be fully evaluated, but there is no doubt that the mass growth of cyanobacteria has never been documented in such detail before. As Figure 3 shows, cyanobacteria proliferated in deeper waters in June, and then, towards the end of July, the species *Microcystis aeruginosa* started to become dominant close to the surface. At the beginning of August, the entire plankton community was dispersed over various depths by cold and windy weather, and after the mixing event the cyanobacteria started proliferating again close to the surface. In the middle of the month, the cyanobacteria bloomed and rose to the surface, where high light intensity appears to have inhibited their production of the pigment phycocyanin. The production of gas vesicles inside the cells, which helped cyanobacteria to reposition themselves in the water column after the mixing event, may have caused their rise to the water surface and the formation of scum observed in Lake Greifen over the same period. Part of the population subsequently returned to its preferred depth of around 6 – 8 metres.

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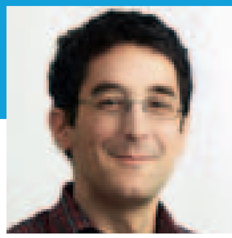
Forum



Florian Altermatt, biologist and group leader in the Aquatic Ecology department, studies species distribution and interactions.

Biodiversity patterns shaped by connectivity

River systems accommodate high levels of biodiversity. While greater species richness is generally seen in lower reaches than in headwaters, the latter exhibit greater variation in species composition. It has now been shown experimentally that diversity patterns of this kind can arise as a result of network structures typical of rivers. Habitat connectivity is thus a key factor in the preservation of biodiversity.

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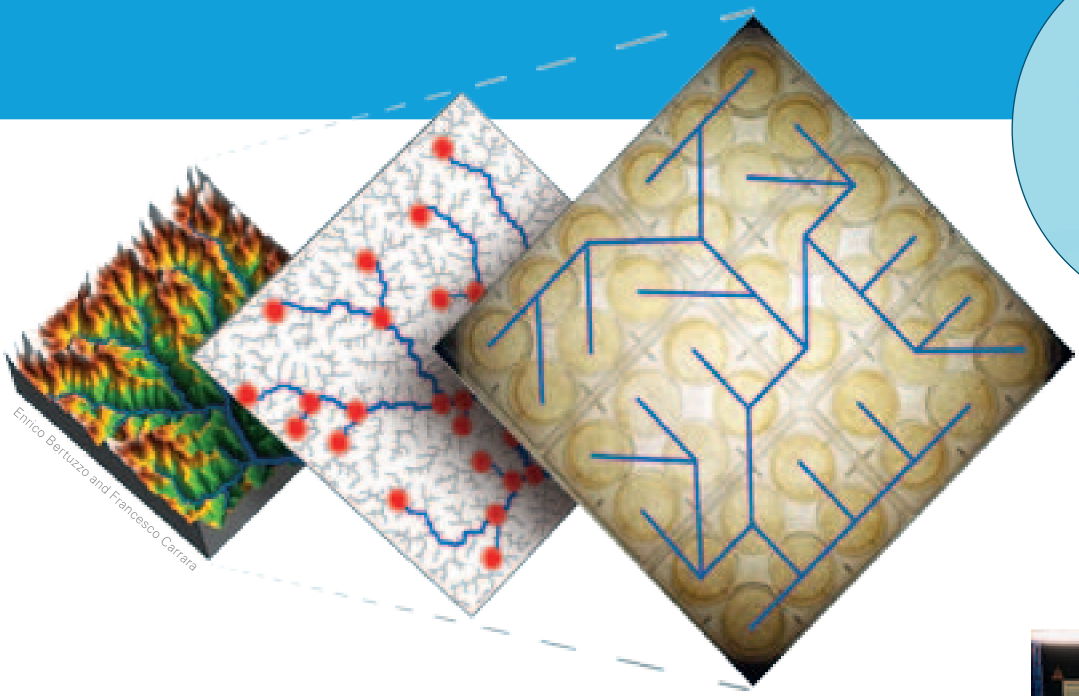
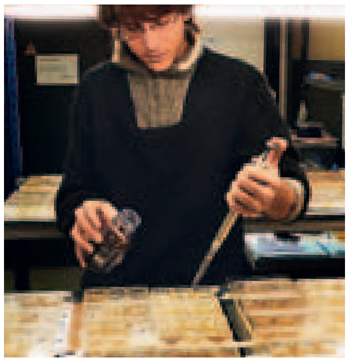


Enrico Bertuzzo and Francesco Carrara

Fig. 1: From landscape model to laboratory system: the river network structure was extracted from a landscape model, and a simplified version was projected onto an aquatic microcosm system. Dispersal of organisms between neighbouring sites followed the structure of the river-like network.

Regula Illi and Florian Altermatt

Some of the microorganisms used in the experiment (protozoans including *Euglena gracilis*, *Colpidium* sp. and *Blepharisma* sp. and the rotifer *Cephalodella* sp.). The individual organisms are between 20 and 100 micrometres in length.



Florian Altermatt

Initially, the same number of species were present in all the culture plates. Pipetting of culture medium allowed organisms to disperse along the network structure. In the course of the experiment, differentiation of local communities occurred.

What factors determine which species – and how many different species – occur in a particular area? Explanations for the occurrence of biological communities observed in nature are not only of scientific interest, but essential for the protection and management of habitats. Various large-scale diversity patterns have been identified, and efforts have been made to define the rules which govern the composition of communities. For example, it is known that more species are found on large islands than on small ones. In 1967, this fundamental principle of biogeography was explained by US biologists Robert MacArthur and Edward Wilson as follows: the colonization of an island by new species and the extinction of existing species are in a state of dynamic equilibrium. The rate of extinction decreases as the area of the island increases, while the rate of colonization rises with increasing area and proximity to the mainland. Accordingly, larger and less isolated islands harbour more species than their smaller and more isolated counterparts [1]. This theoretical concept has practical applications – e. g. when decisions have to be taken concerning the size of protected areas.

Dispersal along waterways

Rivers and streams are among the most species-rich ecosystems worldwide [2]. However, this diversity is widely threatened by habitat degradation and fragmentation, the spread of invasive species and water pollution. To facilitate effective conservation, therefore, a better understanding of the factors underlying biodiversity is urgently required. At the same time, freshwater biologists are also seeking general principles to explain natural biodiversity.

Rivers often show characteristic diversity patterns [3–5]. For example, headwaters are usually less species-rich at the local scale (α diversity) than lower reaches, but they show more marked variation in species composition among communities (i. e. higher β diversity). In the past, the occurrence of species and these typical diversity patterns were largely explained in terms of local environmental conditions; for example, various species can only survive within specific pH or temperature ranges. But this approach fails to take account of the dispersal of organisms; in addition, it assumes that every suitable habitat will be occupied.

However, far from being static, the spatial and temporal distribution of species is a highly dynamic process: organisms can colonize new sites while disappearing from others. Many fish and macroinvertebrates use waterways to transport their dispersal forms. Riparian plants, likewise, shed their seeds into the water and can thus colonize downstream habitats. In such cases, the structure of the river network will determine where they end up. Several centuries ago, the typical branching structure of river systems was described by Leonardo da Vinci as a hierarchically organized, ramified network, resulting from hydrological processes. In ecology, however, such networks were long neglected, with waterways being conceived of as simple linear systems [6]. Only recently has the structure of river networks been taken into account in efforts to explain the occurrence of species [4].

According to current models, the characteristic diversity patterns observed in rivers are due to the fact that organisms have only a limited ability to disperse in a river network [3, 5]. These models take account of the fact that organisms mainly move along the line of flow, that the distances travelled are relatively short, and that species may undergo local extinction in individual river stretches as a result of various processes. Whether habitats are recolonized will depend on their location within the network and on the availability of nearby source populations.

The models thus predict that it is not necessarily or not only local environmental conditions which determine where certain species do or do not occur, but that habitat connectivity also plays an important role. In other words, the specific network structures of river habitats give rise to characteristic diversity patterns. However, while these mathematical models closely reflect the diversity patterns observed in nature, experimental confirmation has so far been lacking.

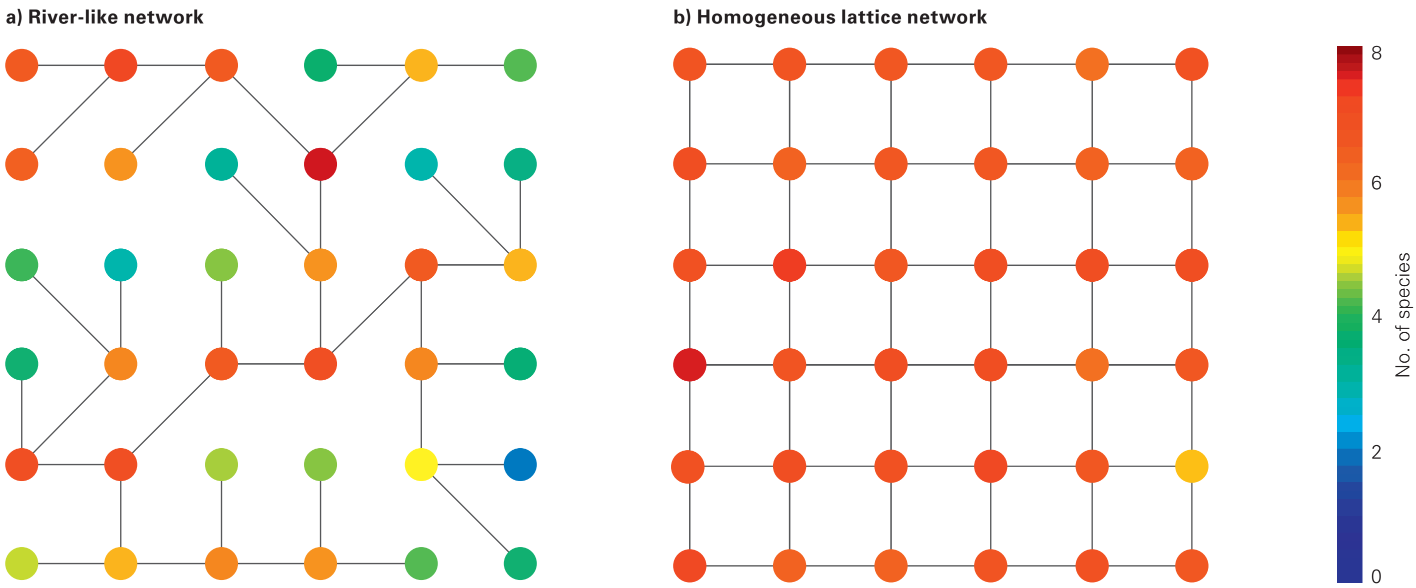
Experimental river network

In a recently published study, we showed for the first time on the basis of a laboratory experiment that the dispersal of organisms along river-like networks leads to characteristic diversity patterns [7]. Thanks to cooperation between Eawag and Andrea Rinaldo and his colleagues at the EPFL Laboratory of Ecohydrology, it was possible to combine empirical and theoretical approaches.

For the experiment, we reproduced a – highly simplified – dendritic river network in the laboratory. The spatial structure of a river network was extracted from a realistic landscape model, and a simplified version was applied to our laboratory system, which consisted of culture plates (Fig. 1). Each local community initially comprised eight protozoan and one rotifer species in a culture medium. To simulate the dispersal of microorganisms within this miniaturized river network, we used a pipette to transfer small amounts of nutrient medium between the various communities. Transfers were made between neighbouring sites along the schematic river courses and, for comparison, along a homogeneous lattice network (Fig. 2). At the end of the experiment, the level of biodiversity was determined for each site.

With the aid of the simplified laboratory system, environmental factors could be either eliminated or harmonized. For example, the dispersal rates and habitat conditions were identical in the two experimental set-ups. The systems differed only in their connectivity, i. e. in the dispersal paths open to the organisms. We were thus able to investigate the causal link between the spatial characteristics of a river-like network and biodiversity. As the experiment covered dozens of generations of the organisms concerned, it was also possible to consider long-term effects of species interactions and population dynamics on diversity patterns.

Fig. 2: Local diversity depends on connectivity. In river-like networks (a), the central "confluences" show higher species richness than the "headwaters". In homogeneous lattice networks (b), the distribution is highly uniform.



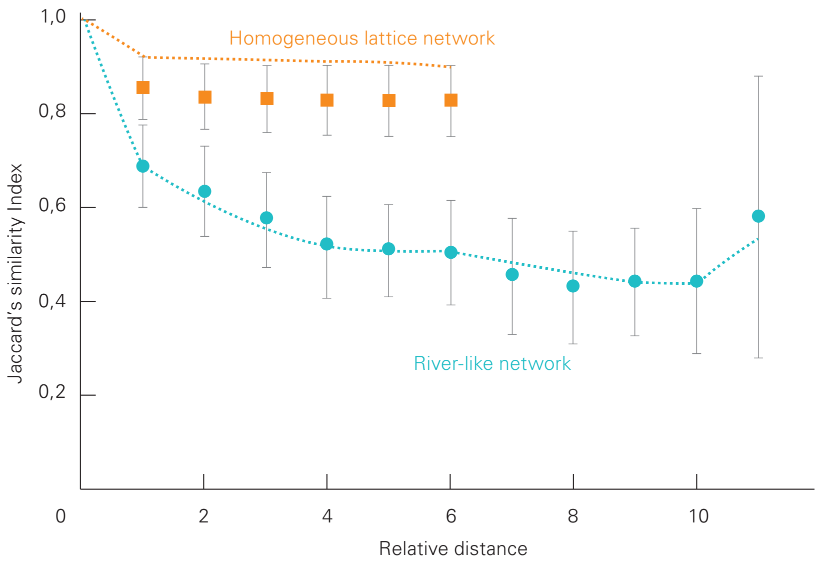


Fig. 3: Pairwise similarity of local communities as a function of distance within the network. The degree of similarity is shown by the Jaccard index. A value of 1 indicates that two communities have the same species composition, while 0 means that they have no species in common. Solid symbols represent the experimental observations (mean ± SD), and dotted lines the model predictions.

Diversity influenced by network structure

In the river-like networks, the "confluences" showed greater species richness at the end of the experiment (after about a month) than the more isolated "headwaters". In addition, there was marked variation in species richness among the various headwaters. In the homogeneous lattice networks, by contrast, a monotonous pattern arose (Fig. 2). In both experimental setups, similarity of species composition decreased with increasing distance between the local communities. Especially in the river-like networks, the greater the distance between local communities, the more they differed (Fig. 3). A mathematical model developed in parallel with the laboratory study confirmed the experimentally observed patterns, allowing us to draw more general conclusions.

The results demonstrate that the connectivity and continuity of rivers have a direct influence on species distribution and diversity. The structure of river systems affects two key indicators of biodiversity – local species richness within habitats (α diversity) and variation in community composition among habitats (β diversity). The latter is vital for the maintenance of a high level of regional diversity.

What are the implications for real-world systems?

Our approach is based on a heavy simplification of natural systems, and we cannot draw any direct conclusions concerning a specific river or particular species. However, the simplification and replication of key factors make it possible to test how river network structure influences biodiversity in principle, as well as yielding more general findings. Our investigations suggest that changes to the network structure of actual river systems can have direct effects on species dispersal and diversity. For example, the construction of dams leads to habitat fragmentation, while channels linking watercourses will increase connectivity. The consequences also need to be taken into consideration in conservation efforts. Thus, when a river section is subject to conservation or restoration measures, attention should be paid not only to local environmental factors – the position of the section within the river network is equally crucial. Isolated headwater habitats are more difficult for species to colonize than central habitats. On the other hand, the interlinking of naturally isolated habitats may facilitate the spread of invasive species.

Our experiments also confirm that river network structure should be included as an important factor in mathematical models designed to predict diversity. This in turn should permit additional predictions on a broader basis. Connectivity parameters can be varied in models so as to compare realistic future scenarios. As part of our research project, we are applying our findings to study the distribution and diversity of aquatic insects in Switzerland's river system. Of particular interest is how the diversity of mayflies, stoneflies and caddisflies can be explained not only by local environmental conditions, but also with reference to the position within the river network.

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Co-author: Beat Müller

Eutrophication in China – déjà vu for Europe

Rapid economic and population growth in the Beijing-Tianjin region is leading to heavy eutrophication of the Haihe river system. Nutrient inputs derive mainly from wastewater. Although treatment plants have been constructed in some areas, these often operate beyond their projected capacity or with poor nutrient removal efficiency.

Benoit Pernet-Coudrier

Algal bloom in the highly eutrophic Shahe Reservoir upstream of Beijing.

Northeastern China is one of the country's most rapidly developing areas. The region incorporating the megacities of Beijing and Tianjin has the highest rates of economic and population growth nationwide. However, this semi-arid region is increasingly suffering from droughts and water scarcity. Since the 1970s, rainfall has decreased markedly. In 2007, water availability in Beijing dropped to 230 cubic meters per inhabitant per year – less than eight per cent of the amount potentially available in Switzerland.

Despite the dry climate, a remarkable 30 per cent of China's wheat and 20 per cent of its maize is produced in the agricultural area between Beijing and Tianjin. This is only possible thanks to intensive irrigation with water from the Haihe river system, which extends from the Shahe Reservoir upstream of Beijing to Bohai Bay near Tianjin (Fig. 1). As a result of the decline in rainfall and the increasing use of water to satisfy the demands of agriculture, industry, a growing population and an increas-

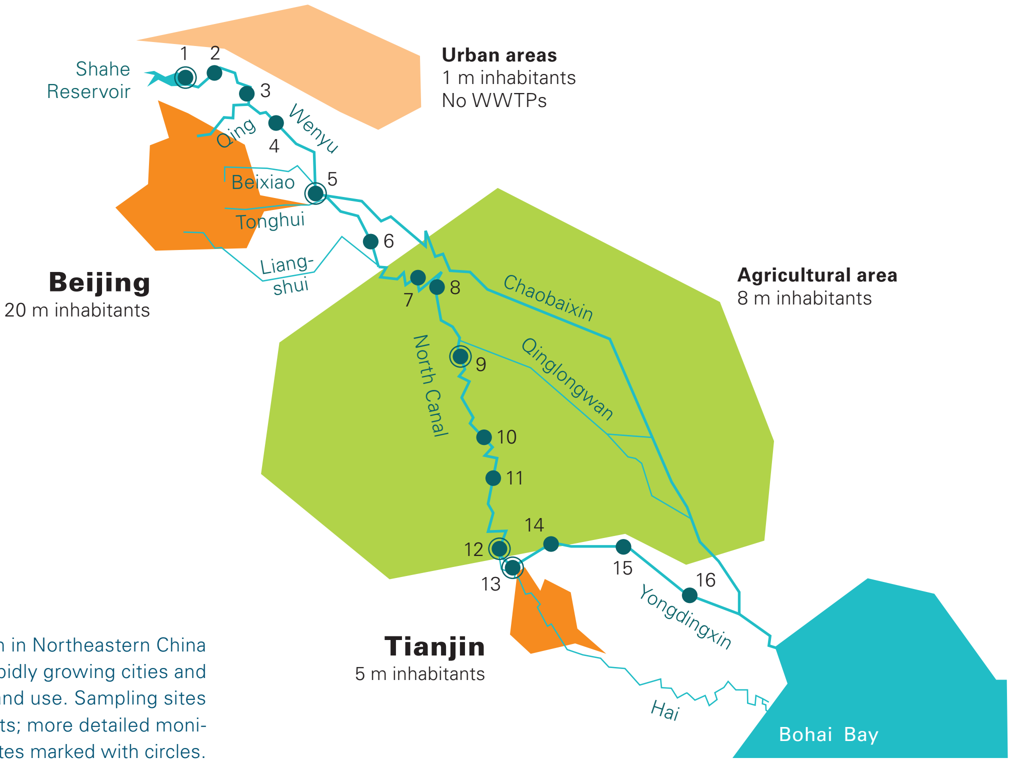


Fig. 1: The Haihe river system in Northeastern China receives pollution from the rapidly growing cities and from intensive agricultural land use. Sampling sites are indicated by numbered points; more detailed monitoring was carried out at the sites marked with circles.

ing per-capita consumption, many rivers in Northern China have now run dry.

Wastewater: the main cause of eutrophication

In addition, over the past 40 years, concentrations of nutrients (nitrogen and phosphorus) in the Haihe river system have risen dramatically. The eutrophication of these surface waters is attributable partly to overuse of chemical fertilizers, but mainly to the fact that a large proportion of wastewater from the cities was discharged untreated. As a result, the waters are now severely degraded; oxygen depletion and algal blooms are widespread. Pollution also enters the soil, groundwater and the sea, with grave consequences: altered nutrient ratios lead to shifts in the composition of marine algal communities. So-called red tides – where algal blooms are produced by species that release toxic substances – are becoming increasingly serious. Parts of China's coastal waters are among the world's largest "dead zones" – oxygen-depleted areas where almost no marine life can survive.

To some extent, Europe experienced a comparable deterioration of the aquatic environment during the boom years which began in the 1950s. In Switzerland – thanks not least to the findings of research on mitigation strategies – this problem had been significantly alleviated by the 1990s, with the adoption of measures such as the construction of wastewater treatment plants (WWTPs) and a ban on phosphates in detergents [1]. Today, similarly intense remediation efforts are urgently required in China and in other emerging economies around the world.

In Northern China, the importance of the water pollution issue has been recognized, and it now ranks high on the political agenda. Funds for mitigation are more readily available than in other nations, and this should facilitate the implementation of effective measures, as well as collaboration with Western experts and knowledge transfer.

High consumption in spite of water scarcity

Before the 2008 Olympic Games, nine new WWTPs came into operation in Beijing, with the goal being to treat 90 per cent of the wastewater discharged from the city into the Haihe river system. To date, however, the effectiveness of these measures has not been evaluated. In a project conducted jointly with the Research Center for Eco-Environmental Sciences (part of the Chinese Academy of Sciences) in Beijing, we therefore sought to establish – for the first time – an overall budget of nutrient fluxes for the Haihe river system [2]. To this end, we assessed the spatial and temporal patterns of nitrogen and phosphorus loads in the catchment, from the Shahe Reservoir upstream of Beijing to Bohai Bay near Tianjin. The aim of the study was to provide a sound scientific basis which would enable the authorities to develop appropriate strategies for the mitigation of eutrophication in the Beijing-Tianjin region. A complementary study, also involving Eawag scientists, investigated the contamination of surface waters with organic micropollutants (see the article on page 6) [3].

To gain an initial overview of the occurrence and abundance of nutrients, we analysed water samples collected at 16 selected sites along a 240-km river section in the dry (April) and wet season (July) of 2009. On the basis of this preliminary assessment, five key sites were selected for further monitoring, with samples being collected monthly for a year (Fig. 1). At these sites, weekly and diurnal variations were also examined. In addition,

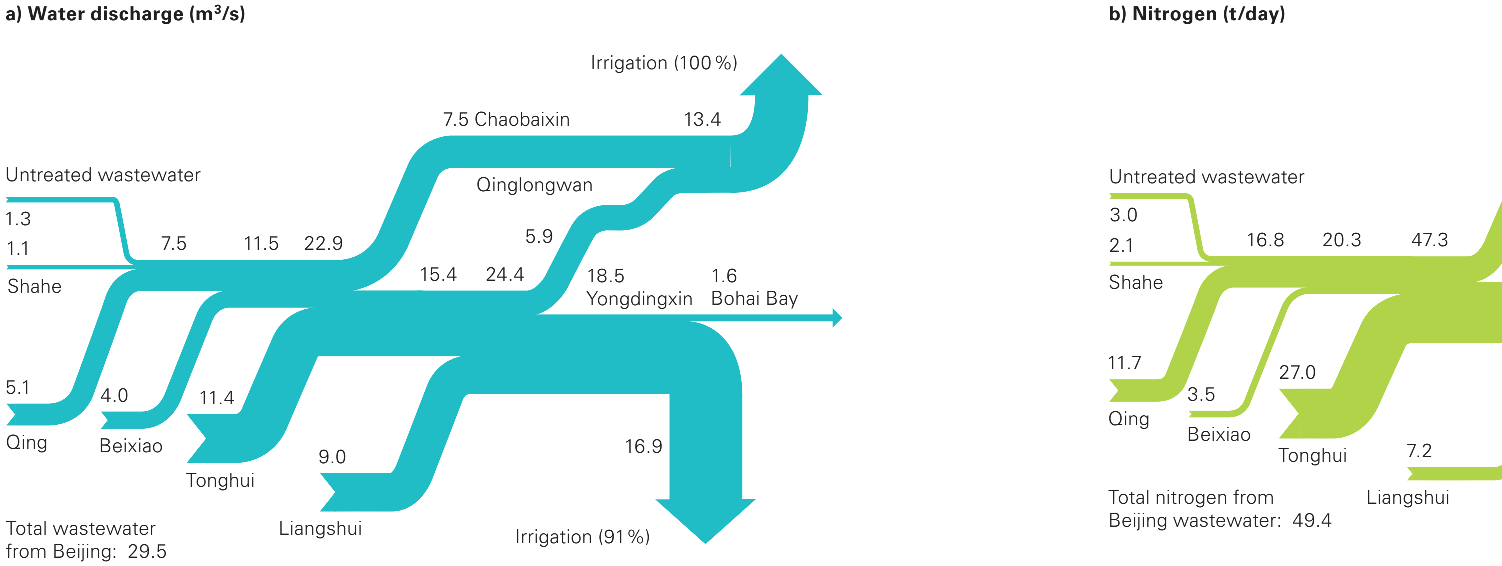


Fig. 2: Water discharge and nutrient loads in the Haihe river system.

the four tributaries (Qing, Beixiao, Tonghui and Liangshui) draining wastewater from Beijing were investigated, and effluents from the city's five largest WWTPs were sampled in order to assess per-capita nutrient loads.

It initially proved difficult to determine water discharge at the various study sites, as the data collected by the local authorities was not publicly accessible. This problem was only resolved thanks to our local partners' persistent efforts and negotiations. The outflow from the Shahe Reservoir – a lake with an area of 1.8 square kilometres situated in a public recreation park – was reported to be a mere trickle of less than 1 cubic metre per second. Downstream of Beijing, the Qing, Beixiao, Tonghui and Liangshui tributaries account for 90 per cent of total discharge. Beijing releases an annual average of 29.5 cubic metres per second of raw and treated wastewater into the Haihe river system (Fig. 2a). This wastewater derives from around 14 million inhabitants, or 70 per cent of Beijing's current population. Daily average water consumption amounts to 200 litres per inhabitant, which means that the region – despite water scarcity – has higher per-capita water consumption than Switzerland (170 litres per day).

Extremely high nutrient levels

Beijing and its suburbs discharge an average of 54.5 tonnes of nitrogen and 4.5 tonnes of phosphorus per day into the Haihe river system (Fig. 2b and c). Wastewater effluents are the major source of both nutrients (over 90 per cent), with the Tonghui river contributing more than half of the total load.

Concentrations of up to 33 milligrams of nitrogen (in the form of ammonium) and 3.2 milligrams of dissolved inorganic phosphorus per litre were observed in the Shahe Reservoir – more

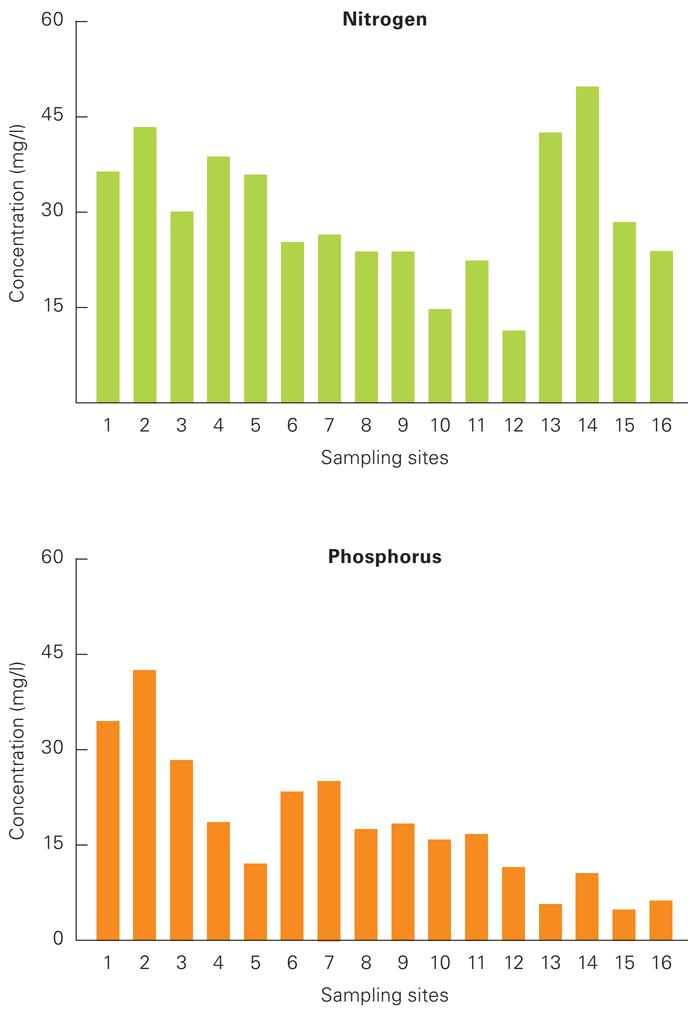
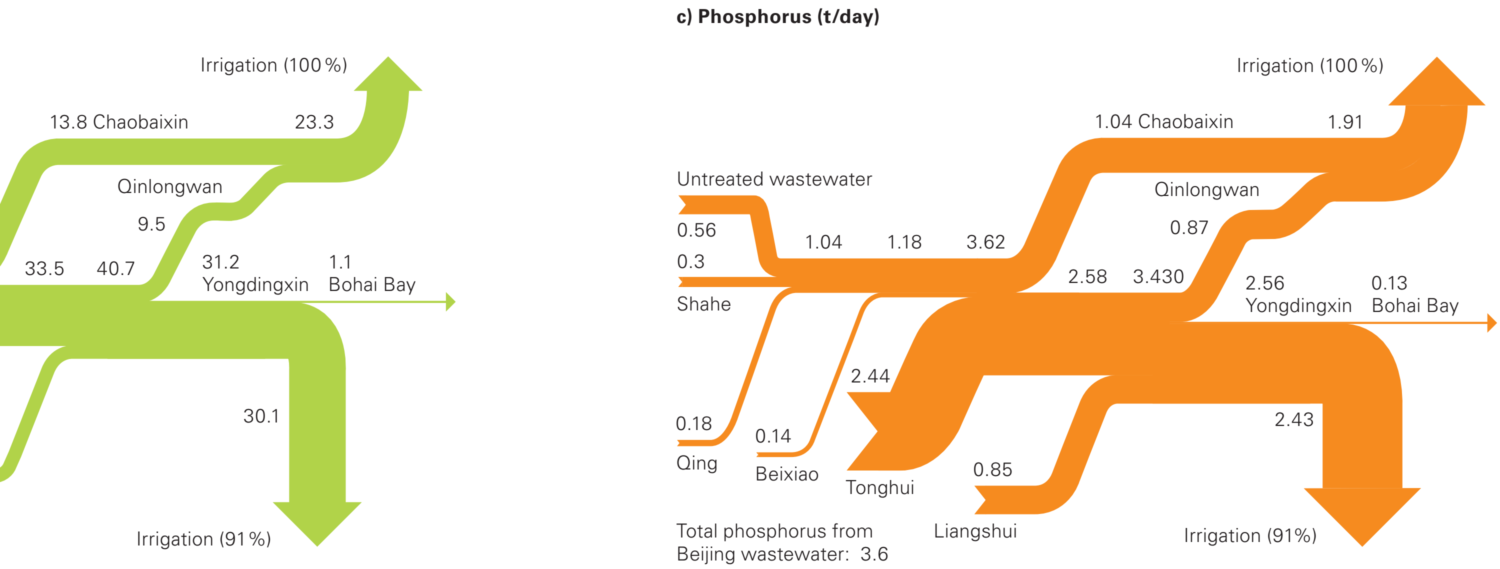


Fig. 3: Nutrient concentrations show a decreasing trend downstream.



than ten and six times higher than the maximum levels seen in Switzerland during the 1970s/1980s, when surface water eutrophication was at its peak. The nutrients derive mainly from untreated domestic sewage and sludge from animal husbandry (especially poultry farming). Large amounts of organic carbon-rich sediments are thus deposited in this shallow lake, continuously releasing nutrients and trace elements into the water column. In addition, downstream of the reservoir, countless small sewers discharge untreated wastewater into the Wenyu river.

Nutrient concentrations are thus particularly high in the upper sub-reach of the Haihe river system, although this region only contributes about three per cent of the overall nutrient load. Further downstream, concentrations of nitrogen and phosphorus decrease more or less steadily (Fig. 3). Nutrient concentrations would normally be expected to increase near urban areas (such as Beijing) and in intensively used agricultural areas (such as the region between Beijing and Tianjin). However, pollution from the Shahe Reservoir is so extreme that nutrient concentrations are diluted by inputs of treated wastewater from Beijing.

Inadequate removal of nitrogen at WWTPs

Overall, our analyses confirm that wastewater is the main source of nutrients entering the Haihe river system. Other sources – industrial and agricultural inputs or atmospheric deposition – appear to be negligible. According to our calculations, 1420 grams of nitrogen is produced per inhabitant per year in Beijing. This is close to the values reported for untreated wastewater around the globe (1650 – 1850 grams). However, the value calculated for treated wastewater – more than 1200 grams per inhabitant – is considerably higher than the values typically reported internationally (110 – 800 grams). The poor treatment performance suggests that nitrogen removal is impeded by sluggish nitrification/denitrification processes at WWTPs.

In contrast, phosphorus removal is relatively efficient: the estimated discharge rate was within the range reported globally for treated effluents – 91 – 211 grams of phosphorus per inhabitant per year. This means that the very high concentrations still observed in some rivers should be attributed to low water levels rather than to inadequate wastewater treatment.

Over the last few decades, despite the construction of nine WWTPs and more than 4000 kilometres of sewers in the Beijing area, it has not been possible to curb the exponential increase in nutrient concentrations in the Haihe river system (Fig. 4). Although increasing numbers of households in Beijing have connections to WWTPs, population growth was underestimated when the plants were planned in the early 2000s; as a result, they are often operating beyond their projected capacity.

In our view, the most effective way of controlling massive eutrophication in the region would be to construct additional WWTPs with sufficient capacity and state-of-the-art denitrification efficiency. Measures of this type made it possible to at least halt the increase in nitrogen levels in Switzerland and Europe in the 1980s – though in many cases nitrogen fluxes today remain far above pristine levels, on account of innumerable non-point sources that are difficult to control.

The Chinese agricultural sector could also help to conserve water resources and energy by adopting a more cautious and responsible approach to the use of mineral nitrogen fertilizers. In particular, this could mitigate eutrophication of coastal regions,

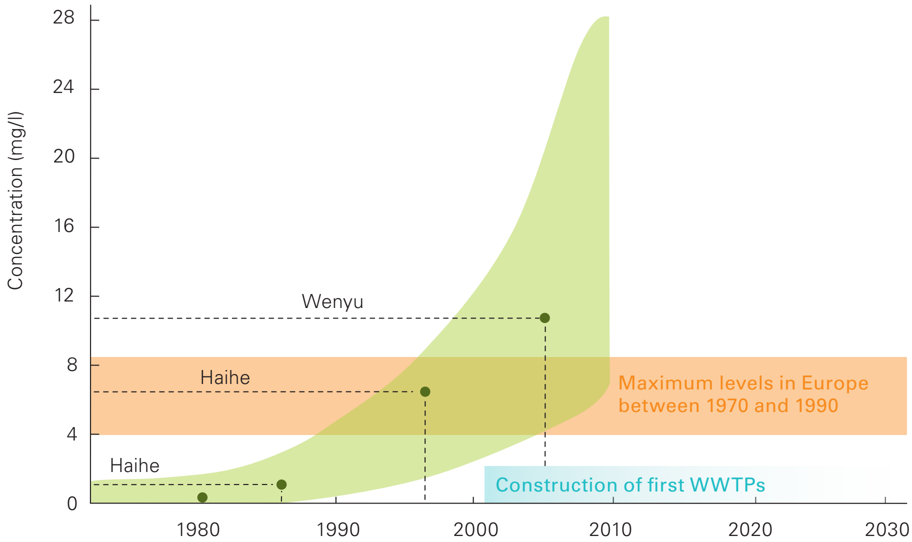


Fig. 4: Nitrogen levels have continued to rise steeply despite the construction of WWTPs, exceeding by a large margin the highest levels ever recorded in Europe.

such as the Yangtze estuary [4]. A ban on phosphates in detergents would also be required if phosphorus levels are to be reduced.

Polluted water used for irrigation

The results of this joint Sino-Swiss study were communicated and discussed at various workshops involving authorities and other stakeholders in Beijing. It was apparent that China is determined to tackle this issue. The authorities have already installed pilot plants for advanced wastewater treatment at five WWTPs in Beijing, with plans to upgrade to full-scale operations in the near future.

The importance of decisive action on this matter is highlighted by another finding of our study: of the annual average of 31.9 cubic metres of water per second which flows through the Haihe river system, around 95 per cent is withdrawn for irrigation. Almost all the water channelled to the Chaobaixin and Qinlongwan rivers is used for this purpose. Of the 18.5 cubic metres of river water per second discharged in the North Canal, downstream of Beijing, less than 10 per cent (1.6 cubic metres per second) actually reaches Tianjin and eventually the sea (Fig. 2a). Several dams along this section of the river divert water to an extensive network of irrigation channels, used by countless farmers. The water used exceeds grade V of the national quality standard – i. e., strictly speaking, it is too polluted to be used for any purpose – and thus poses risks to food safety and groundwater quality.

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Taking the human factor into account

The causes of environmental problems are primarily social. But in environmental research and practice, they still tend to be approached from a purely natural-science and engineering perspective. The integration of a social-science perspective is essential if more sustainable solutions are to be achieved. Though this insight is not new, it still needs to be more widely understood and accepted. *Text: Andres Jordi*


Keystone

Recreation is just one of the many purposes for which water is used (pictured here is the 2012 Zurich lake crossing event).

In environmental research, there is a growing awareness that ecological problems frequently cannot be resolved solely by applying the methods of natural science and engineering. Any approach which essentially focuses on physical, chemical and biological processes will tend to overlook the relevant social systems and stakeholders. "Environmental problems are primarily caused by social factors," says Bernhard Truffer, head of Eawag's new Environmental Social Sciences department. "That means you have to understand the social systems and processes if you want to deal with the impacts." Social-scientific disciplines, he adds, can improve our understanding of the origins, assessment and management of environmental problems.

Who could disseminate on-site wastewater systems?

Heiko Gebauer and Bernhard Truffer investigated the potential role of business networks in the dissemination of on-site wastewater treatment technologies. The question at issue was whether multinational companies (e. g. General Electric, Siemens or Veolia) or small and medium-sized enterprises (SMEs) could promote the spread of these systems worldwide. According to the study, multinational companies are best placed to do so. However, as Gebauer explains, "Barriers are created by the initially low market potential and the fact that companies would cannibalize their centralized wastewater technologies in existing markets." This could be avoided by increasingly shifting system development to emerging economies which lack large-scale treatment infrastructure, and then launching on-site systems as an alternative to centralized technologies. This type of approach is often not feasible for SMEs, which are forced to develop on-site systems for niche markets in industrialized countries. But, so far, the niche markets appear to be too small for costs to be driven down to attractive levels, thus preventing the development of further applications. Both for multinationals and for SMEs, it is important that value-creating activities relating to on-site wastewater systems should be appropriately linked to urban, wastewater and sanitary planning processes [6].
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Dominant role of technology
A prime example of how nature and the environment are closely interlocked with social systems is water. We harness water power to produce electricity, use rivers and lakes for recreational purposes, implement flood control measures, use water resources for drinking water supplies or irrigation, or seek to conserve aquatic habitats and biodiversity. Underlying these activities are not only scientific conceptions and technological solutions, but also – crucially – different human needs, interests and values, i. e. social factors. According to Heinz Gutscher, Professor of Social Psychology and Director of the Social Research Unit at the University of Zurich, "Human perception, thought, decision-making and action need to be understood in the context of families, groups, organizations and society. But debates on, say, future energy supplies have so far been dominated by technology."

The need to approach environmental problems from a social angle is also recognized by practitioners, as is shown by research recently carried out at the University of Bern [1]. According to this study, Swiss conservation practitioners believe that the most pressing concerns are questions relating to economic, societal and stakeholder conflicts, and they wish to see greater integration of social sciences into practical ecology. Similar views are expressed by European water management experts: to ensure the sustainability of services, there is an urgent need for multidisciplinary research, bringing together engineers and social scientists [2].

Damian Dominguez, an engineer in charge of wastewater disposal at the Canton Bern Water and Waste Management Office, is also convinced of the importance of a social-science perspective for practitioners: "Particularly when long-term strategies have to be developed, the social sciences can provide valuable tools for decision-making, for prioritizing goals or for handling complex questions." As an example, Dominguez cites the "Regional Infrastructure Foresight" (RIF) project, in which he was personally involved: this method, developed at

Are the costs of upgrading treatment plants justified?

With the aid of a cost-benefit analysis, Ivana Logar and Roy Brouwer are studying whether planned upgrades to wastewater treatment plants in Switzerland which will permit the removal of micropollutants are economically justifiable. The estimated costs of these upgrades are around CHF 133 million per year. The environmental economists conducted a representative survey in German-speaking Switzerland to find out how much households would be willing to pay for the removal of micropollutants. Logar says: "Our study shows that citizens are well informed about micropollutants. And 54 per cent of the respondents consider them to be risky or even very risky for the environment and human health." Preliminary results on willingness to pay suggest that the removal of these substances from wastewater would be worth more than CHF 200 million a year to the Swiss public. This would mean that the upgrading of wastewater treatment plants is justified from an economic viewpoint. **ivana.logar@eawag.ch**

Eawag, is designed to support strategic decision-making for sustainable infrastructure planning in the urban water sector [3]. RIF provides a methodological framework for the analysis and evaluation of future scenarios and technical/organizational options in a participatory planning process. The results of this process can be used to generate recommendations for more detailed planning steps. The application of RIF in various regions of Switzerland has shown that this method produces better results than conventional approaches, and participants have been very satisfied with the procedure and outcomes. In the RIF process, solutions calling for greater professionalization of wastewater organizations fared much better than those involving incremental developments.

Lengthy process

The idea that social sciences need to be increasingly integrated into efforts to resolve environmental problems is not a new one. As former Eawag Director Ueli Bundi recalls, "Back in the 1970s, it was already apparent that environmental problems affect many areas of society, and that purely scientific and engineering approaches were inadequate." According to Bundi, the impetus for a broader approach also came from practitioners and environmental organizations. These developments are reflected in Eawag's own history. At an early stage, the institute recognized the need to take economic and legal questions into consideration [4]. In 1987, Eawag scientists – contributing to a Swiss Science Council research policy report on early detection in water protection – argued that water research must take account of the interrelationships between scientific, engineering and social-science matters and recommended interdisciplinary collaboration [5]. This led to the setting-up of a Human Ecology research group in 1992 and subsequently to the establishment of an Innovation Research department (Cirus). More recently – in the autumn of 2012 – Eawag's diverse activities in this field were merged to form a new Environmental Social Sciences department (see interview). Examples of the types of questions investigated by social scientists at Eawag are to be found in the Boxes.

How can planning processes be improved?

As part of the NRP61 project "Sustainable water infrastructure planning", Judit Lienert, Florian Schnetzer and Karin Ingold are investigating how a combination of stakeholder analysis and social network analysis can help to assess and improve our understanding of decision-making processes. In particular, this research aims to support the development of future strategies for water supply and wastewater management. Using systematic surveys, the researchers studied the relationships and interactions between the stakeholders and sectors concerned. They found not only that there is little collaboration between the water supply and the wastewater sector, but also that there are few ties between actors at the communal, cantonal and national level. "Infrastructure planning is clearly dominated by engineers and local authorities," says Lienert. The researchers conclude that linking stakeholder analysis to social network analysis provides insights into the complexities of the socio-political-engineering world – a key requirement if instruments for joint planning processes are to be developed [7]. **judit.lienert@eawag.ch**

However, the integration of social sciences into truly interdisciplinary environmental research is a lengthy process. Bundi explains: "Every scientific discipline has its own rules and structures and cultivates different worldviews and conceptions of science; this makes exchanges difficult, because often the partners do not fully understand each other." In addition, he believes the rewards for engaging with other disciplines in conventional scientific activities are insufficient. According to Truffer, "One of the main problems we face as social scientists is the widespread expectation that we are mainly concerned with solving practical problems. Often, our colleagues from the natural and engineering sciences fail to understand that we address research questions of our own and are not merely consultants." Truffer adds that many people have difficulty accepting that in social processes there are no simple mechanisms which can be adjusted in order to resolve problems: "Usually, there's no such thing as a social end-of-pipe solution."

As Dominguez points out, obstacles still remain for social-scientific strategies in practical settings, too: a lot of people continue to believe that all environmental problems can be remedied by technical solutions if the details are sufficiently well understood. Practitioners, he adds, generally have a scientific or engineering background and are not familiar with social-scientific thinking:

New department of Environmental Social Sciences

In September 2012, a new research department was launched at Eawag. As Bernhard Truffer – head of the new organization – explains, the aim of Environmental Social Sciences is to build on the experience Eawag has accumulated in this area over many years.

Why has Eawag established the new Environmental Social Sciences department?
The main aim is to consolidate the skills developed and the experience acquired over the past 20 years. The new department is essentially based on the Cirus (Comprehensive Innovation Research in Utility Sectors) organization, which was set up in 2006, and two other existing research groups. In the last two years, another two groups had been established in the fields of environmental economics and environmental policy, so it was time to bring our social science capabilities together in a new department.

What topics are covered by Environmental Social Sciences?
It's not a discipline taught at universities. We chose this name because the researchers in the new department come from a wide range of social science backgrounds – economics, political science, social psychology, business administration or economic geography. Within each of these disciplines, there are fields of specialization explicitly concerned with environmental topics.

What areas will the new department be focusing on?
Our research is subdivided into five competence clusters – Environmental Economics, Policy Analysis and Environmental Governance, Environmental and Health Psychology, Environmental Innovation and Management, and Decision Analysis. Our priorities will initially be shaped by the establishment and further development of these competence clusters. As regards water-related processes, we'll be focusing on topics such as new technological and organizational options in urban water management, the handling of micropollutants in aquatic systems and the management of environmental resources.

What challenges are involved in collaborating with the natural and engineering sciences?
Over the years, we've gained a lot of experience in inter- and transdisciplinary collaboration, and it generally runs very smoothly. Eawag is widely regarded as a model institution for transdisciplinary research. But you have to be aware that the process of getting to know and value each other takes time. Eawag offers a good environment for ensuring that people can climb this learning curve. In other settings, experiments are often broken off prematurely because of difficulties arising in the early stages. However, it should also be borne in mind that more integration is not always better. Cooperation between a number of disciplines requires, first of all, a high-quality problem analysis, jointly prepared by experts from the various areas. Only then can you decide where particular disciplinary competencies are needed to attain the defined objectives.

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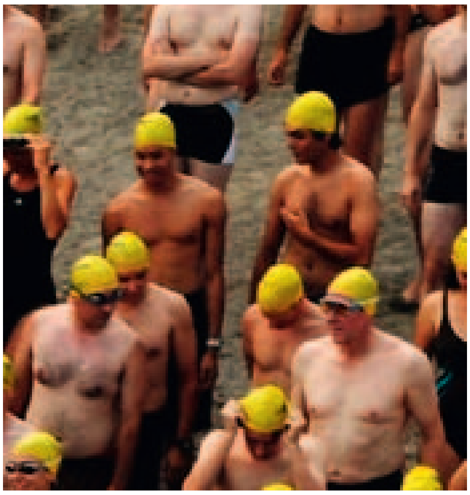

How can handwashing be effectively promoted?

In response to the devastating earthquake and the subsequent cholera outbreak in Haiti in 2010, the aid agency Oxfam carried out public health campaigns designed to improve hygiene behaviour among the local population. The influence of these promotional activities on handwashing behaviour was evaluated by Nadja Contzen and Hans-Joachim Mosler of Eawag on the basis of structured interviews conducted in over 800 households. It was found that whether people wash their hands at key times depends primarily on social norms, basic attitudes to handwashing and individual ability beliefs. In addition, statistical analysis indicated that faeces- and food-related handwashing was effectively promoted by hygiene radio spots, distribution of materials with instructions and demonstrations, information from friends or neighbours, and hygiene theatre. The effects were stronger when promotional measures were combined than when applied individually. In contrast, hygiene songs, focus groups or posters were associated with less frequent handwashing, and special hygiene days or home visits were also found to be counterproductive [8].

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"So unfortunately, rather than asking themselves fundamental questions, they often get bogged down in technical details."

Mandatory for planning procedures

Nonetheless, as the various examples from Eawag's research show, efforts to integrate a social-science perspective can sometimes be fruitful. And over the past 20 years, as Bundi emphasizes, transdisciplinary and participatory approaches have increasingly been adopted in scientific studies and also for planning procedures. Thus, today, they are a mandatory part of the processes specified e. g. for restoration, land improvement, hydropower or flood control projects. Equally, the National Research Programme on "Sustainable water management" (NRP 61) includes not only projects concerned with natural systems but also a "social systems" research axis, dealing with socio-economic changes and cross-sectoral strategies for sustainable management and protection of water resources. Heinz Gutscher, the social psychologist, sees positive signs of change: "At the moment, there's a strong trend for greater involvement of social sciences in environmental topics. Let's seize this opportunity!"

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In brief

Method included in Swiss Food Compendium

The flow cytometry-based method developed by Eawag for the analysis of drinking water has been included in the Swiss Food Compendium. This publication, overseen by the Federal Office of Public Health, is an official compilation of recommended and mandatory analytical methods used for quality control of foodstuffs, additives and articles of daily use. In the assessment of the microbiological quality and safety of drinking water, flow cytometry provides much more realistic results than the legally specified heterotrophic plate count (HPC) method. HPC can only detect bacterial cells which grow on solid nutrient media (agar plates) – around one per cent of all the microorganisms present in a water sample. In addition, flow cytometry is much quicker than the conventional method: results are available after a quarter of an hour, whereas HPC analysis can take several days or even weeks, depending on the individual pathogen. www.slmb.bag.admin.ch

What are the effects of micropollutant reduction?

Eawag is launching an initiative known as "EcoImpact" – a major, interdisciplinary research project designed to study how micropollutant reduction at wastewater treatment plants affects surface water quality and ecosystem functions. Over the next 20 years, Switzerland plans to invest approximately CHF 1.2 billion to upgrade about 100 of the country's 700 WWTPs, with the aim of reducing levels of organic pollutants (from medicines, cleaning agents, personal care products or pesticides) in natural waters. In the initial phase, using laboratory experiments and field studies, researchers will investigate the impacts of micropollutants on the aquatic environment at various levels of biological organization – from cell to ecosystem. The effects of WWTP upgrades on aquatic systems are to be examined in the second phase of the project. Contact: christian.stamm@eawag.ch

First ozonation facility being built in Dübendorf

In October 2012, the foundation stone for Switzerland's first ozonation facility was laid at the Neugut WWTP in Dübendorf. The micropollutant reduction step is to come onstream in the autumn of 2013. Ozonation has been successfully used for decades in drinking water treatment, for disinfection and elimination of taste and odour compounds. Ozone is a highly reactive gas which attacks persistent compounds and can thus eliminate micropollutants. The new facility will provide initial experience of long-term operation. In collaboration with Eawag and other partners, the ozonation process is to be further optimized, and a measurement and monitoring concept is to be developed. www.neugut.ch

Major honour for SODIS project leader

At a ceremony held in Chur on 24 November 2012, Martin Wegelin was awarded the Dr J.E. Brandenberger Foundation Prize (worth CHF 200,000) for his work on solar water disinfection (SODIS). Wegelin – who retired in 2007 after a 25-year career at Eawag – led the team which studied the principle of using sunlight to purify contaminated water in PET bottles, and implemented the method in practice. The Eawag team demonstrated that pathogenic microorganisms are inactivated by the sun's ultraviolet radiation and explored how SODIS can be most effectively propagated. According to the foundation's president, it was thanks to Wegelin's scientific curiosity, commitment and persistence that the SODIS method is now widely accepted and used by around five million people in 28 developing countries. Wegelin had tirelessly travelled the world, establishing partnerships and, in many countries, also gaining the support of the authorities. While Eawag continues to investigate options for the dissemination of SODIS, the project is to be handed over to the development organization Helvetas by 2015. www.sodis.ch

Retirement of Thomas Egli

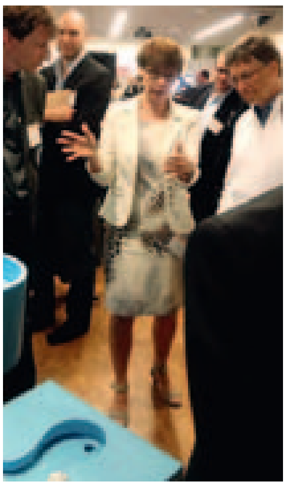
In August 2012, Thomas Egli – formerly head of the Environmental Microbiology department – retired after 30 years' service at Eawag. Among other achievements, the microbiologist played a leading role in the development of a reliable and efficient method of drinking water analysis based on flow cytometry (a technique widely used in medicine), which could well replace existing methods. For this work, he received the 2010 Muelheim Water Award and was honoured by the Swiss Gas and Water Industry Association (SVGW).

New head of Surface Waters department

On 1 September 2012, Carsten Schubert took over from Alfred Wüest as head of the Surface Waters department. Schubert, a biogeochemist, joined Eawag 10 years ago. The research group which he leads studies biogeochemical processes in lakes and reservoirs (e.g. degradation of organic compounds in water and sediments).

New professorships for Eawag scientists

In May 2012, the ETH Board appointed **Alfred Wüest** of the Surface Waters department as Full Professor of Aquatic Physics at the Federal Institute of Technology in Lausanne (EPFL). Wüest, who is already Titular Professor at the ETH Zurich, studies physical processes in lakes, reservoirs and rivers. In September, **Max Maurer** was appointed Full Professor of Urban Water Systems at the ETH Zurich. As head of the Urban Water Management department, Maurer is particularly interested in the engineering potential of innovative approaches to urban water management and innovative management and planning procedures for water infrastructure. Also in September, **Hong Yang** of the SIAM department was appointed Associate Professor of Sustainable Water Use at the University of Basel. Yang's research group studies the impacts of growing water scarcity and food trade on global food security. She is well known for her work on the concept of "virtual water".



Gates Foundation recognition award

The "diversion toilet" developed by Eawag and the Austrian firm EOOS received a special recognition award for outstanding design – and a prize of USD 40,000 – from the Bill & Melinda Gates Foundation. The Foundation's "Reinvent the Toilet Challenge" aims to encourage researchers to develop "next-generation" toilets – innovative sanitation systems operating without piped water, sewer or electricity grid connections, transforming waste into useful resources, and costing no more than five cents per person per day. For project leader Tove Larsen, it was obvious that the model developed for the competition should be based on the principle of separation of urine and faeces: "Separation technology permits efficient recovery of valuable resources and simple recycling of water." The team's squatting toilet is acceptable to various cultures and only requires about one and a half litres of water per use. Water is purified for reuse by means of a membrane filter. The toilet is designed to be part of a total sanitation system, including collection, transport and processing of faeces, with costs being covered by the income generated. The researchers now plan to build and test real prototypes of their toilet. www.eawag.ch/rttc

In brief

Just published

Eawag's report on **Wastewater Management 2025** (published in German, with an English summary) reviews existing knowledge and seeks to identify gaps in our knowledge of the current situation and the future of wastewater management in Switzerland. The emphasis is placed on bringing together the meta-knowledge available at the national level and identifying future action areas. Special attention is paid to the direct and indirect effects of climate change on wastewater management. http://library.eawag-empa.ch/schriftenreihe/schriftenreihe_21.pdf

In a new volume entitled **Chemistry of Ozone in Water and Wastewater Treatment: From Basic Principles to Application,** Eawag scientist Urs von Gunten and co-author Clemens von Sonntag discuss mechanistic details of ozone reactions and apply them to micropollutant degradation. As well as providing sound foundations for researchers, the book offers information in a compact form for practitioners responsible for the planning or operation of ozonation steps in drinking water and wastewater treatment plants. www.iwapublishing.com

The multidisciplinary "Integrated watershed management" programme provided a scientific basis for the planning and implementation of river restoration projects. The collection of information leaflets on **River engineering and ecology** published by the Federal Office for the Environment (in French/German/Italian) presents the results of the project, jointly conducted by Eawag, the Federal Institute for Forest, Snow and Landscape Research (WSL) and the Federal Institutes of Technology in Zurich and Lausanne. It is addressed to experts working in federal and cantonal agencies, as well as engineering and environmental consultancies. www.bafu.admin.ch/publikationen/publikation/01678

Treatment of source-separated domestic wastewater is the subject of a new book edited by Eawag scientists: **Source Separation and Decentralization for Wastewater Management.** In this volume, new technologies and management concepts are described, and the advantages and challenges of these approaches are discussed by researchers and practitioners. www.iwapublishing.com

Factsheets and publications

On its website, Eawag publishes Factsheets on a variety of topical issues. Recent additions to the series deal with "Water and energy", "Hydropower and ecology" and "Rainwater usage". **www.eawag.ch/medien/publ/fb**

A database of publications by Eawag researchers is available online. Open access publications can be downloaded free of charge: **www.lib4ri.ch**

Courses

26 February 2013 **Eawag Dübendorf**	Der Einsatz von umweltpsychologischen Massnahmen für Verhaltensänderungen im Umweltbereich
6 March 2013 **Eawag Dübendorf**	Wasserwissen – die effiziente Informationsrecherche
10–12 April 2013 **Eawag Dübendorf**	Planning and Design of Sanitation Systems and Technologies
23 April 2013 **Eawag Dübendorf**	Stoffflussanalyse und Modellierung
7 May 2013 **Eawag Dübendorf**	Revitalisierung von Fliessgewässern: Ansätze für die Priorisierung
7 June 2013 **Eawag Dübendorf**	Drinking Water Quality in Developing Countries: Mitigating Geogenic Contamination
11–12 June 2013 **Eawag Dübendorf**	Einführung in die Ökotoxikologie
19 June 2013 **Eawag Dübendorf**	Auswirkungen der Re-Oligotrophierung auf Ökologie, Artenvielfalt und Funktion der Seen

Events

22 March 2013 **Stade de Suisse, Berne**	Wasser überwindet Grenzen – Beispiele und Chancen in der Schweiz
16–19 June 2013 **ETH Zurich**	8th IWA Specialized Conference on "Assessment and control of micropollutants and hazardous substances in water"

Further informationen: www.eawag.ch/veranstaltungen