Joint Danube Survey 3

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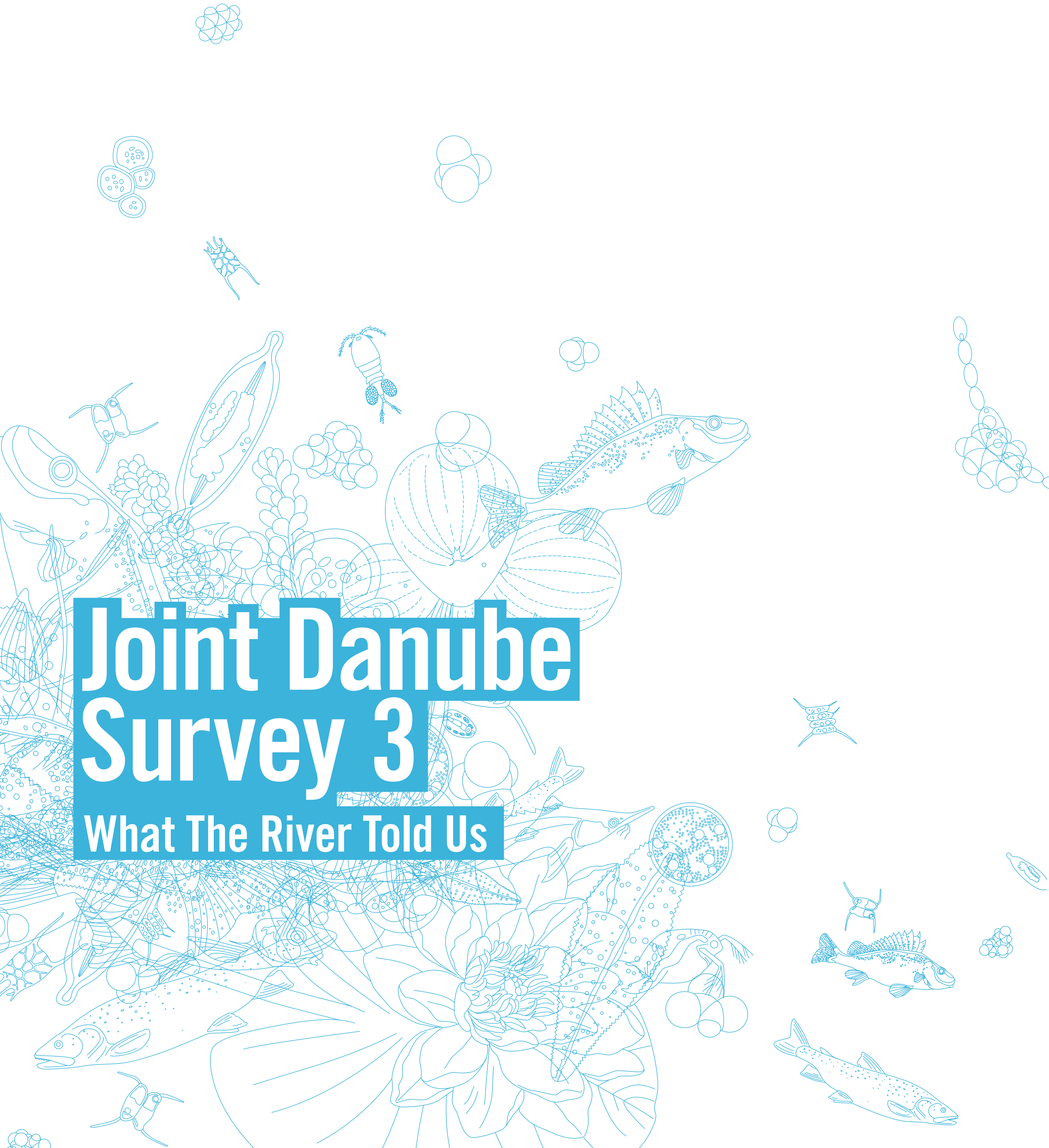
International Commission for the Protection of the Danube River
Internationale Kommission zum Schutz der Donau



Joint Danube Survey 3

What The River Told Us

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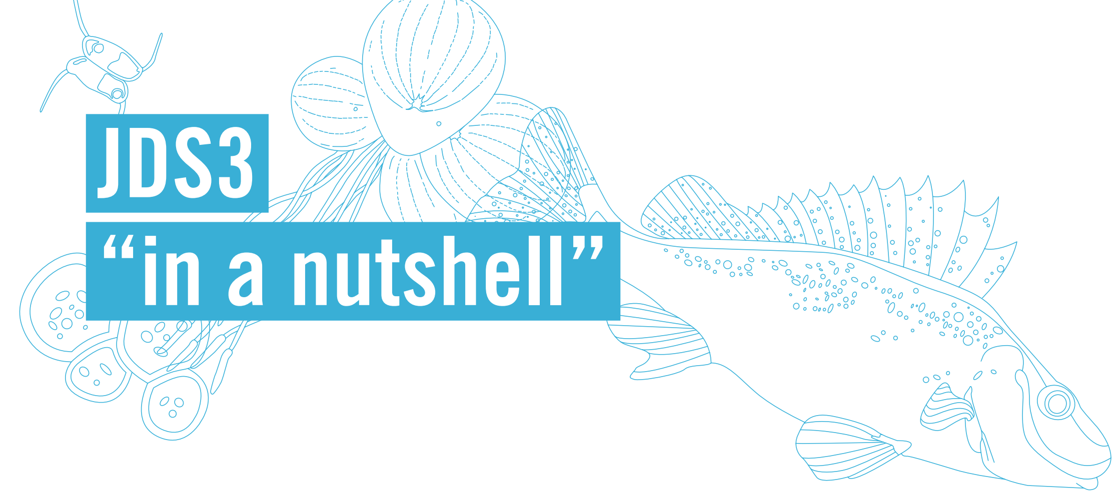
Note: Many of the terms in this report are technical in nature. The Glossary at the end provides readers with helpful explanations and definitions.

Covering more than **800,000** square kilometres and **10% of continental Europe**, the Danube River Basin extends into the territories of **19 countries**, making it the **most international** river basin in the world. The **Danube River** is divided into **three ‘reaches’**. The **Upper** Danube stretches from the Danube’s source in Germany to the ‘Porta Hungarica’ east of Vienna. The **Middle** Danube then flows until the Iron Gate dam in Romania. The **Lower** Danube then runs into the Black Sea.



JDS3 “in a nutshell”

What is the Joint Danube Survey 3?

The *Joint Danube Survey 3*, known as *JDS3*, was the world’s biggest river research expedition in 2013. The JDS is carried out every six years – JDS1 was in 2001 and JDS2 in 2007. For six weeks between August and September, the JDS3 ships travelled 2,375 km downstream the Danube River to the Danube Delta, assessing the Danube and many of its tributaries.

The JDS3 catalyzed international cooperation from the 14 main Danube Basin countries and the European Commission. It was coordinated by the International Commission for the Protection of the Danube River (ICPDR). An international JDS3 Core Team of 20 scientists was responsible for sampling, sample processing, on-board analyses and all survey activities. JDS3 National Coordinators facilitated organization at the national level. Chemical analyses were carried out by leading laboratories across Europe. Corporate partners, such as the Coca-Cola System and Donauchemie, also supported the JDS3.

Why is the JDS3 so important?

In 1994, Danube Basin countries signed the *Danube River Protection Convention (DRPC)* to work toward joint management of water in the Danube River Basin. In 2000, the EU-wide *Water Framework Directive (WFD)* came into force, establishing a legal framework to protect and enhance the status of aquatic ecosystems, prevent their deterioration and ensure the long-term, sustainable use of water resources. In response, Danube countries, including non-EU Member States, agreed to implement the WFD throughout the entire Danube River Basin, with ICPDR coordination.

The WFD requires all EU surface inland waters, and transitional and coastal waters, to achieve ‘good chemical *and* ecological status (or potential)’ and all groundwaters good chemical and quantitative status by 2015. ‘Good chemical status’ basically means that the water should be clean. However, it is not enough for a river to only have clean water without anything living in it. That is why the WFD also requires ‘good ecological status’ whereby waters must provide good conditions, such as migration routes and suitable habitats, for animals and plants to live healthily. For example, many fish need natural sand bank habitats for spawning, but this may not be available along an engineered stretch of river even though that stretch might have ‘clean water’.

The WFD also required countries to develop a *River Basin Management Plan* (RBMP) by 2009 and to identify ‘measures’ they should take to achieve good status by 2015. Once completed, the *Danube River Basin Management Plan (DRBMP)* addressed four *Significant Water Management Issues*:

1. Pollution by **organic substances**, caused mainly by the emission of wastewater from cities or towns, industry and agriculture.
2. Pollution by **nutrients**, whereby levels of nitrogen and phosphorous get too high in the environment, resulting in impacts such as a lack of oxygen.
3. Pollution by **hazardous substances**, such as man-made chemicals, metals, oil and pharmaceutical drugs.
4. **Hydromorphological alterations** – *or* man-made changes to the shape, structure or flow of a water body – caused mainly by hydropower generation, navigation and flood protection.

The JDS1 and JDS2 expeditions provided essential scientific information to help to identify the main issues in the region and their causes. They assisted decision-makers in selecting the right measures for the Danube RBMP. New species were discovered, such as the floating fern *Azolla*. A new database of over 10,000 photos of the river’s structures was produced. And new techniques and technologies were tested – many of which could improve the work of scientists across the globe.

The *JDS3* is so important because it followed up on this past work to determine if the ‘status’ of waters had improved or deteriorated, as some key measures had already been put in place by ICPDR Member Countries. The JDS3 helped to raise awareness for water protection and the work of the ICPDR – through active communications, media relations and nine public events during the expedition (visit www.danubesurvey.org). And lessons from the JDS success story have inspired international action across the globe, by helping to start up similar freshwater surveys in the Tisza river basin, Danube Delta and the Orange-Senqu River Basin in southern Africa. JDS3 results are now feeding directly into the next update of the DRBMP.

What was tested during the JDS3 and how?

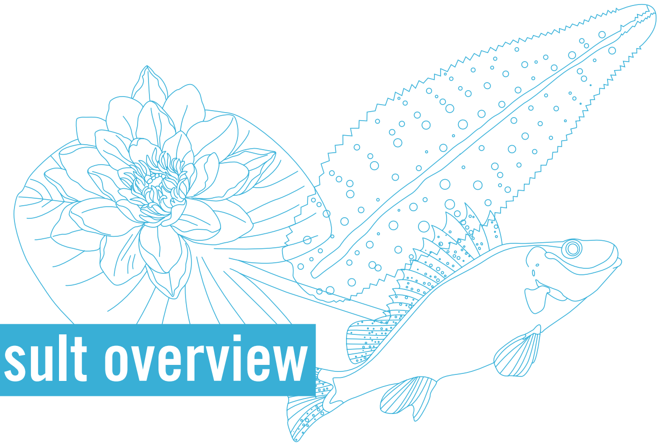
Two ships led the expedition. First, Serbia's *Argus*, the main laboratory ship during both previous surveys, had been recently refurbished and included instruments such as a centrifuge, sieving machine, and microscopes. Second, Romania's *Istros*, a coastal and river research ship with six cabins, a lab and dining room, served as a support vessel. Two Austrian vessels, the *Wien* and *Meßschiff IV*, did the fish sampling.

A total of 68 sites were sampled, with one or two sites visited daily on average. Many samples were tested on-board the ships while others were sent to participating laboratories throughout Europe, within and beyond ICPDR Member Countries.

Sampling at JDS3 stations could include up to five different 'sample types' – water, sediment, biology, suspended particulate matter (SPM) and biota (fish). The experts conducted numerous tests, looking for animals and plants, from larger shellfish to microscopic bacteria, and chemical and hazardous substances. They monitored *physico-chemical* parameters such as temperature and pH. The hydromorphological study included activities such as sediment testing, photography, and listing harbours and sand bars.

32 sites were chosen for monitoring fish. Non-lethal 'electro-fishing' stunned fish for collection. The river bottom was sampled with an 'electrified bottom trawler net'. Experts also removed blood and liver from fish to study the effects of chemicals on living organisms.

Finally, the data and information gathered during and after the expedition was organized into three separate and interrelated assessments – biological, chemical, and hydromorphological – as well as this public brochure.



Result overview

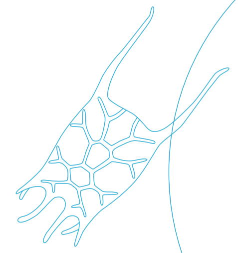
The JDS3 provided the **largest** volume of knowledge about the Danube River Basin ever collected through a single scientific exercise. Overall, the results of the **three interrelated JDS3 assessments** again confirmed that **cooperation** in the Danube River Basin continues to reap rewards. The waters and life within them are progressively becoming **healthier and safer** for all. However, **some problems** still require **measures** to solve them.

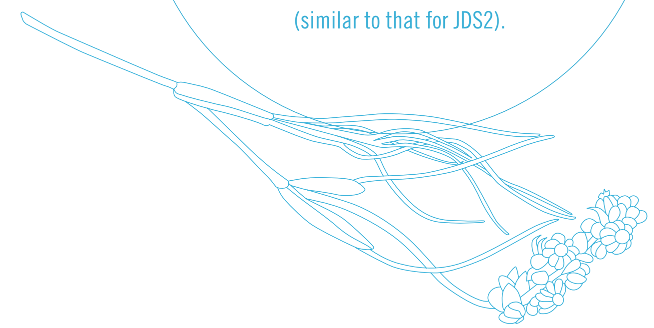
Biological assessment

Of the sites sampled, 77% were classified as having **good or high ecological status**, based on benthic macro invertebrates, especially in most of the Upper and Lower reaches of the Danube. Moderate status was found mostly in the Middle Danube.

The JDS3 reconfirmed that Danube plants and animals show a **high degree of biodiversity**, including a high diversity of **fish**, with over 139,000 individual fish and 67 species sampled. However, due to pressures, such as hydropower, poaching and fishing, about 50% to 90% of the sites did not meet the ecological requirements of the WFD for fish.

Invasive alien species continue to have a constant impact on native wildlife, such as alien fish depleting the habitat of native Danube fish.





Is it safe to swim?
Assessing the safety of swimming in the Danube, according to EU legislation, requires regular long-term monitoring conducted at the national level. However, a quick snapshot from the JDS3 shows good potential for swimming at about 75% of the monitored sites (similar to that for JDS2).

Chemical analysis: key findings

Nutrient (i.e. nitrogen and phosphorus) concentrations in the Danube River declined since earlier JDSs. This and other positive findings may indicate that recent improvements to municipal wastewater treatment are having a positive impact on Danube water quality.

The levels of **metals** in samples were similar to those observed during earlier surveys.

WFD **priority substances** are groups of hazardous chemicals which are of major concern for European waters. While most of the priority substances analysed during the JDS3 were found to be below levels of concern, **some exceeded** them. For example, concentrations of perfluorooctansulfonic acid (a new priority substance that repels water and oil and is resistant to heat and chemical stress) exceeded WFD levels at 94% of sampling sites. WFD levels were also exceeded at some sites for polycyclic aromatic hydrocarbons (occurring in oil, coal and tar deposits as by-products of incomplete combustion processes) and tributyl-tin (used, for example, in anti-fouling paint on ship hulls to prevent the growth of organisms).

Emerging organic substances are chemicals containing carbon which have not been detected previously in water, or which were detected at levels that may be very different than expected. They are not listed on the WFD list of priority substances. Many of these substances were found in the Danube Basin, but at very small concentrations, and usually at lesser concentrations than observed during the JDS2. Some, such as the artificial sweetener acesulfame, were detected in **wells** along the river where water is naturally cleaned by river banks and abstracted by communities for drinking, although levels were non-threatening.

During the JDS3, several **new analytical techniques and strategies** were applied targeting hundreds of organic substances. These resulted in the most comprehensive information ever acquired on this topic for the Danube. For example, *passive sampling* continuously sampled pollutants for several days, to detect small concentrations missed by traditional *spot sampling*. And *biomarkers* assessed how pollution damages DNA in cells.

Hydromorphological assessment

JDS3 findings were similar to those of the JDS2. About **60%** of the entire Danube is slightly or moderately modified. The remaining 40% is extensively or severely modified. No stretches are near natural. Regarding the three **Danube reaches**, the Upper Danube is generally poor, with 75% of stretches intensively altered. About 63% of the Middle Danube proved to be good or moderate. The Lower Danube is generally good and includes the river's longest free-flowing stretch, at 860 km.

The main **problems** are impoundments (e.g. the Gabcikovo and Iron Gates dams), engineered or regulated stretches, dense urbanization, and hydropower. Very few stretches provide good conditions for **floodplains**: the river lost 65%–70% of its floodplains in the past, while many remaining floodplains still suffer (e.g. through disconnection from the main river).

More measures needed

The JDS3 findings confirm that there is still a need for more **measures**. This includes the further construction and upgrade of **wastewater treatment plants**, especially in the Middle and Lower Danube areas, and the implementation of effective policies to reduce **hazardous substances** and **nutrients**. Further research is needed on **invasive alien species** and for the protection of **bank-filtered water wells** used for drinking water production. And the restoration of river hydromorphology and re-establishment of floodplains is required. The ICPDR is working towards coordinating these measures through the updated *Danube River Basin Management Plan* which will be adopted in December 2015.

For more detailed information about the JDS3, visit www.danubesurvey.org.

JDS3

Map of Route and Sampling Sites

The Danube has three 'reaches'
The Danube River is divided into **three** main sections or **'reaches'** – Upper, Middle and Lower. The **Upper** Danube is from the Danube's source in Germany ① to the 'Porta Hungarica' ②, where the Alps' eastern foothills connect with the Carpathian Mountains below the confluence of the Danube and Morava rivers east of Vienna.

The **Middle** Danube flows from the 'Porta Hungarica' to the start of the southern Carpathian and Balkan mountains before the Iron Gate hydro-electric power plant ③. The **Lower** Danube is defined by the Romanian and Bulgarian lowlands including the catchments of the Prut and Siret rivers and their surrounding mountainous landscapes.

Projection: ETRS89-LAEA

This ICPDR product is based on the Joint Danube Survey (JDS3) data. EuroGlobalMap data from EuroGeographics was used for all national borders except for AL, BA, ME where the data from the ESRI World Countries was used; Shuttle Radar Topography Mission (SRTM) from USGS Seamless Data Distribution System was used as elevation data layer; data from the European Commission (Joint Research Center) was used for the outer border of the DRBD of AL, IT, ME and PL.

Based on the Map prepared by Zoran Major, TE GIS ICPDR Produced by ICPDR, Vienna, January 2015

JDS3 Final Scientific Results

Biological Assessment

The biological assessment had **separate assessments** for each of these parameters:

1. **Macroinvertebrates**
2. **Macrophytes**
3. **Phytobenthos**
4. **Phytoplankton**
5. **Fish**
6. **Zooplankton**
7. **Invasive alien species**
8. **Microbiology**

The first five parameters are known as **'biological quality elements'.** Under the WFD, they must be taken into account when assessing 'ecological status'. Special attention was also given to zooplankton and invasive alien species, and to having a separate microbiological investigation.

More coordination needed
The WFD leaves Member States to define their own assessment system. The methods currently in use for the assessment of large rivers in the Danube countries therefore vary. To assess this situation, during the JDS3, different biological monitoring methods were used in parallel, often led to differing results. This showed that more coordination is needed to achieve a more common approach and more harmonized methods throughout the Danube River Basin.

Important biological terms
The following terms are often used to define various aspects of species in this section:
Composition: The identity of all the different organisms that make up an ecological community.
Dominance: The species that predominates in an ecological community, particularly when they are most numerous or form the bulk of the biomass.
Abundance: The number of individuals per species.
Density: The number of individuals of a species in an area.
Diversity: The number of species within a biological community (also known as "richness").
Biomass: Biological material derived from living or recently living organisms.

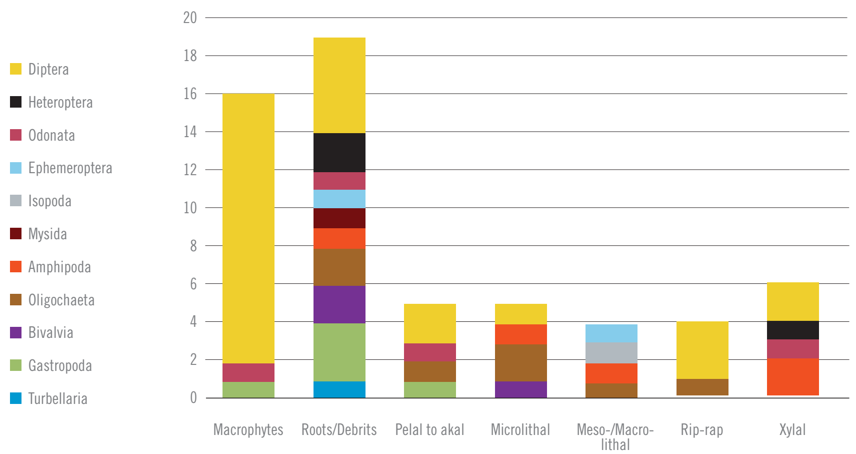
1) Macroinvertebrates

Aquatic insects, worms, clams, snails and other animals without backbones that can be seen without the aid of a microscope and that live in or on sediments.

Introduction
Macroinvertebrates are commonly used to assess the quality of rivers, largely because of the good existing knowledge of their environmental needs and responses to different environmental factors, such as temperature and pollution.

Results
Samples were taken at 55 sites along the Danube stretch. Overall, 460 macroinvertebrate taxa (groups of one or more populations of organisms) were identified. Insects dominated with 319 taxa. Diptera (true flies and midges with a single pair of wings) were the richest insect order with 222 taxa – 200 species belong to the family *Chironomidae*.

Figure 1: Significant indicator species per substrate type



In terms of abundance, Amphipoda (an order of crustaceans known as freshwater shrimp) were the dominant group in all Danube reaches and increased downstream. Diptera play an essential role in the Upper Reach and decreased downstream. Oligochaeta (worms) and Mollusca (like clams and snails with soft bodies and usually a hard shell) were found in increasing numbers in the Middle and Lower reaches, with the invasive Asian clam, *Corbicula fluminea*, occurring in high densities. Asian clams can release up to 2,000 juveniles per day and more than 100,000 in a lifetime.

Regarding water quality, in total, 73% of the 55 sampled sites can be classified as having an 'indication of good ecological status', 15% with an 'indication of moderate ecological status', and 4% with 'high ecological status', according to the WFD. This proportion is similar to that for the JDS2. Serious organic pollution was identified upstream Novi-Sad. Poor status was indicated at these sites: Jochenstein, upstream Drava, downstream Velika Morava, and at Vrbica/Simjan in the Iron Gate reservoir.

Natural (organic) habitats were found to have a higher diversity of animals compared than inorganic habitats, especially artificial ones such as rip-rap where invasive crustaceans are widespread **(figure 1)**. Furthermore, scientists found that the occurrence of species is determined mainly by habitat – information that is important for habitat restoration efforts.

The amazing mayfly

Since the middle of the 18th century, the spectacular mass emergence of the mayfly species *Ephoron virgo* has been reported in the Danube in mid-August. As larvae, these mayflies dwell in the sediment of rivers for months. As adults, they swarm and mate, lay their eggs, and die soon after. Adjacent bridges, ships and roads become covered by millions of these insects which are attracted by artificial light. In former times, the biomass of dead animals was so high that corpses were collected and used as food for swine and poultry. Mass emergences in the Danube had not been observed for decades downstream from the German border, probably due to water pollution and the degradation of their loose gravel habitats. During the JDS2, mayflies were only found at the German stretch between Geisling and Jochenstein.

During the JDS3, they were collected at the German stretch, and surprisingly at all of the Slovakian sites downstream from Gabcikovo and in most of the Hungarian stretch, but not in Austria. The impressive mass emergence was observed only in Deggendorf and Esztergom/Sturovo, although emergences occurring elsewhere may have been missed. Hungarian colleagues reported an emergence in the centre of Budapest a few days before the JDS3 ships arrived. During the JDS3, larvae and adults were also detected downstream Budapest indicating the existence of continuously connected populations over large stretches in the Upper and Middle Danube section. Hence, their recovery can be seen as the result of recent improvements in their habitat quality – nourishing the hope that other threatened Danube species will also return!

2) Macrophytes

Aquatic plants, either free-flowing or attached to the bottom, which can be determined by the naked eye without the need for a microscope.

Introduction

Macrophytes comprise all water plants. They include: vascular plants (those having tissues for conducting water and minerals throughout the plant) such as *angiosperms* (flowering plants) and water ferns; non-vascular plants, or *bryophytes*, such as mosses and liverworts; and macroalgae, such as green algae.

Macrophytes have many benefits. For example, near the shore they help to reduce shoreline erosion by absorbing wave energy. They trap particles and nutrients that can form substrate (surface on which a plant lives) for organisms that then serve as fish food. They provide feeding and breeding habitats and hiding places for aquatic animals, fish, songbirds, amphibians, reptiles and mammals. Macrophytes are also a good indicator for water quality.

Results

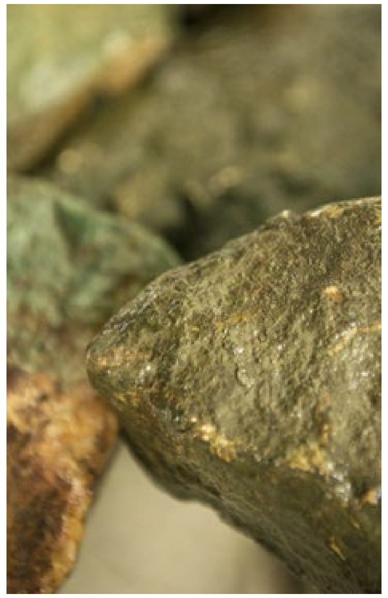
A total of 198 taxa (182 species divided among 16 genera) were identified. These included bryophytes (35 taxa), ferns (4 taxa), angiosperms (150 taxa), charophytes (a division of green algae) (1 taxon) and other macroalgae (8 taxa). Across all of the three JDSs to date, the total number of identified taxa is 249.

From mosses to macroalgae

The composition of macrophyte communities changes along the Danube. Mosses dominate in upper sections where water is colder and fast-flowing and the river bed is covered with hard substrate which is a more suitable surface for them to live on. In lower parts of the Danube, angiosperms start to dominate because the river bed is covered with soft sediments, the bank slope is more flattened, and flow velocity is very low, sometimes near standing. Here we can find floating, emerged and submerged water plants. Macroalgae are present all along the Danube stretch, but are often represented with different species.

Bank marathon

To complete the macrophyte analysis, a scientist had to trawl three kilometres on both banks of each site to assess plant occurrence and diversity. He also walked through 360 km (nearly the same distance from Vienna to Budapest by car) of banks of the Danube River and its tributaries to investigate water plants.



3) Phytobenthos

Microscopic plants such as algae that live in the surface layers of the river and seabed.

What are primary production and chlorophyll?

Primary producers, including phytobenthos and phytoplankton, use sunlight, water, chlorophyll and carbon dioxide to synthesize (combine) organic compounds. In other words, they produce biomass from inorganic compounds. They are thus very important in water bodies as a key source of food and energy for many other organisms up the food chain, and because they produce oxygen. Assessing them helps to indicate the degree of *biological production* in a water body which is a key component of water quality (i.e. high production means high quality). Assessing them is also useful for evaluating the impacts from nutrient pollution, chemicals, or changes to hydromorphology.

The analysis of *chlorophyll-a* (a green pigment found in plants and cyanobacteria) is an essential parameter used to indicate the quantity of algae in water. This is also a quick test of water quality – low values mean low biomass and good water quality; high values mean bad water quality.

Methods

For phytobenthos sampling and chlorophyll-*a* measurements, a river segment with a suitable substrate had to be chosen, along with stones in the current. Stones were measured in-situ for chlorophyll-*a* concentrations using special equipment that take 'fluorescence fingerprints'. Three algal groups were investigated: diatoms (a major group of algae), green algae and cyanobacteria. In addition, material from a small area on each stone was brushed into containers for later lab analyses of species composition.

Results

The highest level of chlorophyll-*a* was detected in the Upper Danube down to station JDS10 (rkm 1895) and then again at sites downstream from JDS40 (rkm 1107).

The results indicate increasing downstream pressures on, and thus general degradation of, the aquatic environment. The ecological status of the Upper Danube varied between high to good, while sites downstream from Budapest appeared consistently below the good/moderate boundary. However, the results of this assessment method are only indicative.

Bloody rocks?

Among the more than 20,000 red algae found world-wide, only a few hundred species live in freshwater – in rivers, springs or on the surface of wet rocks or soil. In the Danube section from Germany to Serbia, three taxa of red algae were found during the JDS3:

(1) *Bangia artropurpurea*, identified in Austria, attaches to stones in rivers with high flow and creates long brown-red filaments;

(2) *Hildebrandia rivularis* was recorded from Kelheim, Germany to Gabcikovo, Slovakia – evidence of good water quality for this river stretch. It forms red or red-purple flat crusty patches on rocks that can look like spots of blood, and can be found in fast-flowing rivers and mountain streams;

(3) *Thorea sp.* was found on hard artificial substrate in the confluence of the Sava River with the Danube. Looking like a brush with branched olive-green filaments, it occurs rarely in mountain rivers and streams, so its presence in a large river like the Sava is unique.

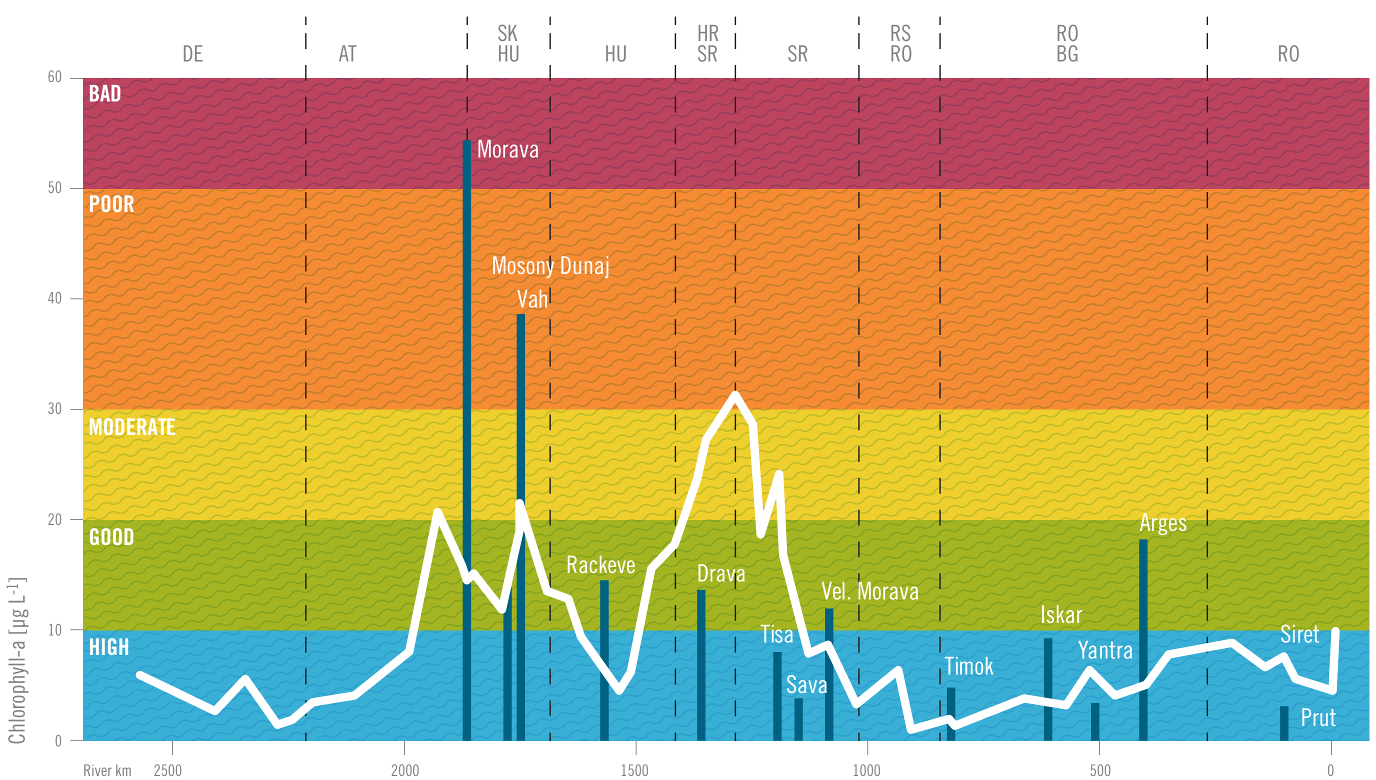
4) Phytoplankton

Plants, mainly microscopic, living in the water column of water bodies.

Results

Chlorophyll-*a* concentrations were measured to assess water quality. Most concentrations in the Danube belonged to water quality class I (high). Moderate values of class II were observed at three sites from rkm 1367 (downstream Drava) to rkm 1262 (upstream of Novi Sad). When applied to river sections, the results indicated high to good status (water quality class 1 – 2) in most of the Upper and Lower Danube (**figure 2**). Moderate status was assigned to the river section from rkm 1384 (upstream Drava) to rkm 1216 (upstream Tisa). The 15 investigated tributaries are in high to good status except for two tributaries.

Figure 2: Chlorophyll-a concentrations in the River Danube and selected tributaries obtained during JDS3, August/September 2013 related to WFD criteria proposed by Mischke and Oppitz (2005)



5) Fish

Aquatic vertebrates (having a backbone) that are typically cold-blooded and covered with scales.

Introduction

Fish are important as a food source and for recreation. Fish populations are also a good indicator for human pressures, especially hydromorphological alterations. *(see more under Invasive Species)*

Methods

The Core Team sampled the near-shore area by electric fishing and the river bottom using an electrified benthic frame trawl net. National teams focused on additional electric fishing in the near-shore zones and different sampling methods at some sites.

'Electric fishing', which was done day and night, is the most used non-lethal method worldwide to sample fish in small rivers or shallow waters. The 'electrified benthic frame trawl' consisted of a stainless steel frame (2m × 1m) with a 5 metre-long drift net. Weighted metal wheels were attached to the frame to keep the device just above the bottom, to prevent the net from filling with material.

Results

In total, 139,866 individuals representing 67 fish taxa were caught which means that the Danube River is an ecosystem with a wide range of fish species. Two species, *Alburnus alburnus* and *Neogobius melanostomus* (or Round goby), dominated with 46% and 26% of the total catch, respectively.

The electrified benthic frame trawl added valuable information which would have remained hidden using only shoreline surveys. For example, it could detect the monkey goby (*Neogobius fluviatilis*) for the first time in the Austrian section of the River Danube. It also revealed the common occurrence and high abundance of Zingel species, especially of *Zingel streber* which occurred at 16 sampling sites with 127 individuals (the JDS2, without the electrified benthic frame trawl, could not prove the occurrence of *Z. streber* in the Hungarian river section of the Danube).

WFD ecological status

Based on fish studies, three national WFD assessment systems were applied during the JDS3, with ranges of 50%, 72% and 93% of sites showing a value worse than "good" and thereby not meeting WFD requirements. No sampling sites showed a high ecological status during either the JDS2 or JDS3. However, as the systems have inconsistent results and react on different stressors, they can apply only to restricted river stretches.

In the Upper Danube, fish results mainly reflected hydromorphological alterations and damming as the most important human impacts, as well as the lack of connectivity. The excessive use of waterpower in the Upper Danube, which leads to degraded aquatic habitats, can be detected easily by the absence of sensitive fish species and certain age classes. The Lower Danube seems to be influenced by professional and recreational fishing and poaching.

6) Zooplankton

Tiny invertebrates (animals without backbones) that float freely in water bodies.

Introduction
Zooplankton are an important part of the pelagic (neither close to the bottom nor near the shore of a water body) food web. They are the main link between small phytoplankton and larger carnivores, primarily young fish.

Small is beautiful
Rotifera, one of the types of zooplankton sampled, have a ring of *cilia* (small hair-like organs on the surface of some cells) which carries food to their mouths and provides them with propulsion. Living mainly in freshwater and some marine waters, they are among the smallest multi-cellular animals.

Results
In total, in the Danube River and its tributaries, 149 zooplankton taxa were discovered – a bit higher than the total in JDS1 and JDS2. These included 107 Rotifera, 33 Cladocera and 9 Copepoda. Along the Danube River, Rotifera and Copepoda were most numerous.

In the Danube River, the density of zooplankton, influenced often by water velocity and turbidity, varied substantially: an increase in density was observed in the slow-flowing Middle Danube reach.

FISHY PROBLEMS
A big issue is fish. One, a majority of the JDS3 monitored sites did not meet the WFD requirements for fish, even though there was no deterioration compared to 2007. Two, mercury concentrations in all fish samples significantly exceeded WFD acceptable levels. Three, invasive alien species are increasing with a constant impact on native plants and animals – for example, the dominance of *Neogobius* fish species in the Upper Danube has dramatically increased since the JDS2, especially in banks artificially protected by rip-rap (large boulders). **A call for action for fish is therefore needed, including more research on the safety of eating Danube fish.**

7) Invasive Species

Non-indigenous species: non-native, alien or exotic.

Introduction
The Danube River is part of the Southern Invasive Corridor which links the Black Sea with the North Sea basin *via* the Danube-Main-Rhine waterway including the Main-Danube Canal. Thus, the Danube River is part of one of the main routes for the migration of aquatic organisms in Europe, including non-native species. As a result, the river is exposed to high potential pressures from biological invasions.

Results
In total, 25 (4 aquatic) neophytes (a plant species recently introduced to an area), 34 non-native aquatic macroinvertebrates, and 12 non-native fish species were recorded.

Most **neophytes** are aggressive and fast spreading. For example, *Robinia pseudoacacia* (Black locust) is adaptable to environmental extremes such as drought, air pollutants and high light intensities. It is also an aggressive, thorny pioneer species and presents a threat to native river vegetation. Among macrophytes, *Vallisneria spiralis* was the most abundant neophyte. Also known as Tape Grass or Eel Grass, *V. spiralis* has flowers carried on long spiral stalks that break away from the plant and float on the water's surface.

Out of 34 **non-native macroinvertebrates**, crustaceans are the most numerous, with 19 species. Eight alien Molluscs and four annelids (a type of worm) were also recorded. Some species considered non-native for the Upper and Middle Danube are native for the Lower Danube. 22 taxa were identified as being of Ponto-Caspian origin (from the Black, Caspian or Azov Sea), spreading from the Lower to the Middle and Upper Danube. Taxa of North American (4), Asian (4), New Zealand (2) and Indo-Pacific (1) origin were also identified. The most abundant and frequent non-native macroinvertebrate taxa along the entire Danube were Crustaceans of Ponto-Caspian origin, such as *Corophium curvispinum,* and Molluscs of Asian origin, such as *Corbicula fluminea* (Asian clam).

Magnificent find
During the JDS3, the North American freshwater species *Pectinatella magnifica* (also known as Magnificent bryozoan, or Moss animal) was recorded for the first time in the main course of the Danube. First detected in the Rackeve-Soroksar Danube side-arm in 2011, the species rapidly colonized a 900 km-long stretch of the Danube between rkm 1586 (downstream Budapest) and rkm 685 (Romanian-Bulgarian stretch of the Danube).

Of the 12 **non-native fish** species recorded, 8 were found in the Upper Danube, 9 in the Middle, and 4 in the Lower Danube. As with macroinvertebrates, the most numerous (5) were fish species that are non-native for the Middle and Upper Danube and of Ponto-Caspian origin. Species of Asian (4 taxa) and North American origin (3 taxa) were also recorded **(figure 3).** The JDS3 found that Danubian fish are heavily influenced by non-native species which were found in all habitats, some in large densities, and some close to the river bottom.

For the entire Danube River, the proportion of alien species to the total fish catch increased from 19.9% to 24.95% from 2007 to 2013 **(figure 4).** New fish species can cause negative impacts on local species due to new parasites and disease, and drastic changes to fish communities and food chains because of increased predation or competition for food. Danubian fish stocks are declining and many species are at the edge of extinction.

Neogobius explosion
The dominance of *Neogobius* species in the Upper Danube dramatically increased since the JDS2. They had high or even dominating numbers along the rip-rap protected banks in the Upper and Middle courses, while downstream the Iron Gate, their abundance was much lower where rip-rap is infrequent. During both the JDS2 and JDS3, *N. melanostomus* (Round goby) was found to be highly dominant outside its natural range of occurrence with a proportion of the total alien catch of 56.7 % during JDS2 and 92.8% during JDS3!

During the JDS2, only 3,389 *N. melanostomus* were caught outside their range of natural occurrence, compared to 31,491 in the JDS3. The proportion of the second most abundant alien species, *N. kessleri,* declined from 20.9% to 1.8%.

The Iron Gate Dam had a big effect on the presence of alien species. Between 2007 and 2013, the proportion of alien species more than doubled from 18% to 37% at sampling sites upstream from the migration barrier, whereas downstream a decrease from 2.6% to 0.3% was detected. Compared to the 34,800 alien specimens caught upstream of the Iron Gate Dam during JDS3, the 137 specimens caught downstream was remarkably low.

Overall level of bio-contamination
The overall situation of bio-invasions over the period 2001-2013, based on the results of four Danube Surveys (JDS1, ADS 2004, JDS2 and JDS3), was assessed. The results clearly show a constant influence of alien species on native plants and animals and a considerable rise in the number of non-native aquatic macroinvertebrate species. Newcomers, such as *P. magnifica*, can occupy a considerable area over a short time period. During the JDS3, 22 more alien macroinvertebrate species were recorded compared with the JDS1. At the same time, non-native species are less dominant in the JDS3 in comparison to the JDS2, which resulted in a lower level of bio-contamination in 2013.

More work needed on aliens
The JDS3 results show that biological invasions in the Danube River Basin should be properly managed. Further work should be done in collecting basic information on the distribution of alien species and their influence on native biota, developing effective tools for assessing pressures caused by bio-invasions, and designing appropriate solutions. However, it is also important to keep in mind that the influence of an alien species on native biota is not always negative. For example, the shells of dead Asian clams that deposit on the river bottom were found to provide suitable habitats for native macroinvertebrate species, such as *Theodoxus* spp. and *T. Transversalis,* an IUCN red list endangered species. Such depositions in the Lower and Middle Danube often occur in deep river zones dominated by pure sand where previously only a few animals could survive.

Figure 3: Mean percentage participation of native and non-native fish species within the three main Danube sections

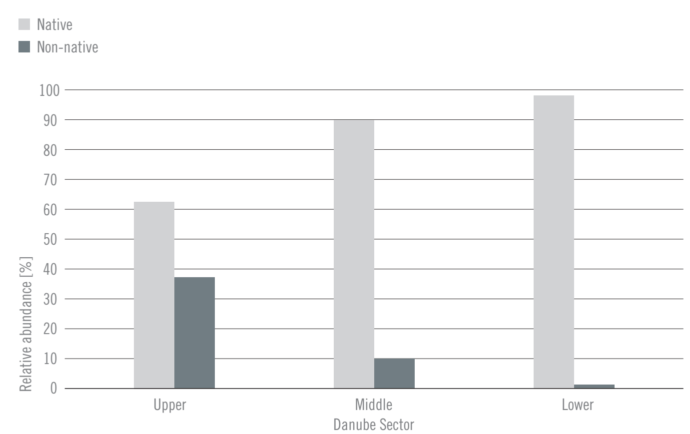
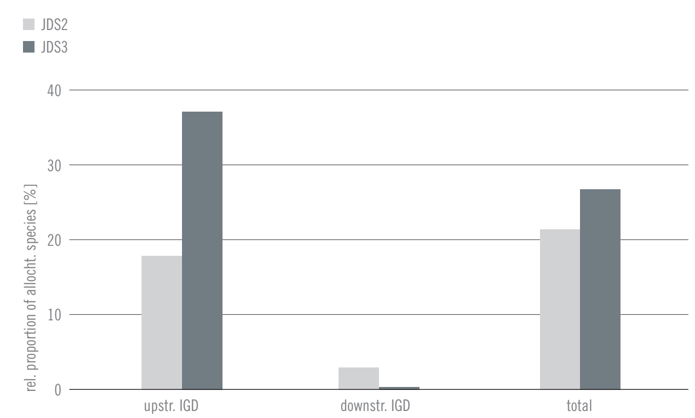


Figure 4: Proportion of alien species to the total catch for the entire Danube River and sections up-and downstream the Iron Gate Dam (IGD)



8) Microbiology

The study of microorganisms – microscopic organisms that are unicellular or exist in cell clusters.

Introduction

The JDS3 microbiological studies focused on assessing the levels and sources of bacteria, as well as bacterial characteristics, such as their resistance to pharmaceutical drugs.

The Danube River and its tributaries regularly receive incompletely treated wastewater from urban areas, animal farms and pastures. These frequently contain faeces which are excreted by humans and warm-blooded animals in high concentrations and which survive for a while in aquatic systems. Faeces frequently contain pathogenic microorganisms like bacteria, viruses and parasites and therefore pose a serious risk to humans. In response, strict quality regulations exist for water intended for irrigation, bathing, aquaculture and human consumption. For example, according to the WFD, designated bathing waters must fulfil the requirements of the EU Bathing Water Directive.

Detailed knowledge on the extent and origin of microbiological faecal pollution is crucial for watershed management activities to maintain safe waters. To assess faecal pollution in the aquatic environment, and the potential presence of pathogens, *Escherichia coli (E.coli)* and *enterococci* are used worldwide as 'indicators'.

Results

Bacterial faecal indicators: Out of 186 total sampling points, 42 (35 Danube and 7 tributary/branch) were classified as critically (34), strongly (5) or excessively (3) polluted by bacterial faecal indicators. The Arges and Russenski Lom tributaries were hotspots of excessive pollution. Surprisingly, Kelheim, Germany, had the highest contamination in the Danube River, although no clear source was identified. Other hotspots with strong or critical pollution levels were the stretch between Novi Sad and downstream Belgrade, downstream Budapest, Dunaföldvar (Hungary), downstream Zimnicea (Romania), and downstream Arges (Romania). Overall, similar values were found during the JDS2.

Microbial faecal source tracking: Given that bacterial faecal indicators cannot show the origin of faecal contamination, microbial faecal source tracking is used. Here, DNA is extracted from water samples for laboratory analysis. During the JDS3, 53 samples from the Danube and 16 samples from tributaries were taken. The results clearly show that human faecal impact is the main driver for faecal pollution levels in the Danube and its major tributaries, as they were in the two earlier JDSs. Faecal pollution from ruminant animal (e.g. cow) and pig contamination did not play a significant role.

Antibiotic resistance: Bacteria that are resistant to antibiotics have existed since the use of antibiotics began. However, recently, the spread of multi-resistant bacteria, outside the hospital environment, has enhanced the problem. One possible transmission route is via wastewater and the water environment. For the JDS3, samples were taken at six sites. A number of antibiotics were later tested in labs on two types of bacteria: *Escherichia coli* and *Pseudomonas spp*. As a result, more than 50% of the *Escherichia coli* showed resistance, although most were resistant only against one or two antibiotics. Hence, multi-resistant bacteria were rare. However, the frequency of multi-resistance (e.g. resistant against up to seven antibiotics) increased downstream. The data therefore shows that Danube waters represent a reservoir for antibiotic-resistant bacteria, especially in the downstream countries.

Chemistry Assessment

This section presents highlights from the **JDS3 Final Scientific Report** related to:

1. **Nutrients**
2. **Hazardous substances**
   (2.1) Heavy metals
   (2.2) Organic compounds

1) Nutrients

Food or chemicals that an organism needs to live and grow, or a substance used in an organism's metabolism which must be taken up from its environment.

Introduction

Plants and microbes need naturally existing levels of nutrients, especially nitrogen (N) and phosphorous (P), to live and grow. However, human activities can result in making the level of nutrients in the environment too high, causing *nutrient pollution* and possibly an unhealthy ecosystem. Nutrient pollution is caused mainly by emissions from cities and towns, industry and agriculture. Deposits from the atmosphere are also a big source, as are many industrial facilities, especially in the chemical sector. Nutrient pollution can cause *eutrophication* – a process where algae and other forms of plant life grow excessively, producing a lack of oxygen and big reductions in water quality, fish and other animal populations.

Eutrophication has impacted many Danube Basin waters and the Black Sea North Western shelf. For the period 1988-2005, the Danube was estimated to carry on average about 35,000 tonnes of phosphorus and 400,000 tonnes of inorganic nitrogen into the Black Sea each year. Presently, the total level of nutrients in the Danube River system is much higher than that in the 1960s, but it is lower than in the late 1980s. The good news is that the ecological situation in the North Western Black Sea coastal area has improved since the early 1990s.

Nutrients from agriculture and detergents

The agricultural sector is a main source of nutrient emissions, especially from mineral and organic fertilisers and livestock manure. Nitrates in particular leach easily into water from soils that have been fertilised with mineral fertilisers or treated with manure or slurry. While the use of fertilisers dropped significantly after the economic collapse in the early 1990s in almost all Danube countries, new measures could become necessary to prevent a rise of pollution in the future. Limiting phosphorus in laundry and dishwasher detergents is also seen as a cost-effective and necessary measure to reduce nutrient pollution.

Results

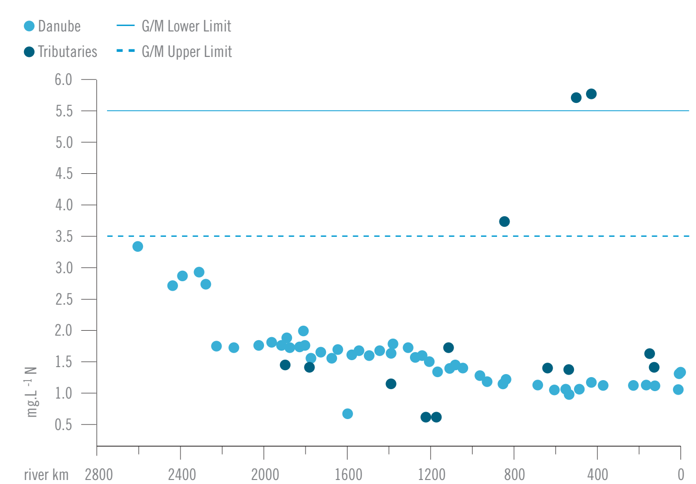
During the JDS3, **Total Nitrogen** (TN) decreased significantly from the Upper to the Lower Danube. The highest levels were at the first five stations. The Iron Gates reservoir had low levels. All the tributaries sampled in the Upper and Middle Danube sections had concentrations lower than in the Danube, except for the Velika Morava. Very low concentrations were found in the Tisa and Sava tributaries. In the Lower stretch, four tributaries – Iskar, Jantra, Siret and Prut– had slightly higher values than in the Danube. Elevated levels were measured in the Timok, Russenski Lom and Arges, most likely caused by insufficiently treated wastewater discharge **(figure 5).**

No trend in **Total Phosphorous** (TP) concentrations along the Danube River was found; still, a slight decrease appeared in the Lower stretch. TP was more pronounced in the Iron Gates reservoir due to its retention of suspended material on which phosphorus accumulates. Six tributaries – Arges, Siret, Iskar, Prut, Jantra and Russenski Lom – had higher concentrations in their confluence with the Danube than in the main course of the river, but no influence on the downstream stretch of the Danube was noticed.

Compared with JDS1 and JDS2 results, TN and TP concentrations measured in the Danube River during the JDS3 were lower, demonstrating a general improvement. JDS3 TN concentrations in the Russenski Lom and Arges tributaries were lower than those in the previous two surveys, while JDS3 TP concentrations in these tributaries were higher. In all three JDSs, TN and TP levels measured in the three arms of the Danube Delta showed that the contribution of the Danube Delta to nutrient retention (plants are able to absorb and retain nutrients, and therefore reduce nutrient pollution) is small – therefore, most Danube water passes directly to the Black Sea.

Ecologically speaking, most sampling sites on the Danube River belong to either "high" or "good" class, except for the Rackeve-Soroksar dammed side-arm (Hungary) and the Iron Gates reservoir area which are "moderate", due to oxygen depletion through the increased degradation of organic pollution. "Moderate" class is also present in several tributaries – Morava, Tisa, Velika Morava, Jantra, Russenski Lom and Arges – caused by low oxygen and high nutrient levels.

Figure 5: Total Nitrogen concentrations in water samples during JDS3 in the Danube River and selected tributaries



2) Hazardous substances

Substances that pose a level of threat to people, the environmental, or property.

Introduction
Hazardous substance pollution can remain in the environment for a long time, seriously damaging river ecosystems and impacting water status and human health, even in low concentrations. Examples include man-made chemicals, metals, oil, and pharmaceutical drugs. Sources of hazardous substances are discharges from industries, storm water overflow, pesticides and other chemicals applied in agriculture, discharges from mining operations and accidental pollution, and deposits coming from the atmosphere.

'Priority substances' are hazardous substances of major concern for European waters. The EU Water Framework Directive (WFD) maintains a list of priority substances that was first set in 2008 by the Directive 2008/105/EC (with 33 substances). The assessment of **chemical status** under the WFD requires an analysis of these priority substances, each of which has an **environmental quality standard (EQS)**, or acceptable concentration level. Assessing status also requires the analysis of additional **non-priority** pollutants discharged in significant quantities that are identified by Member States.

In 2013, a new Directive (2013/39/EU) amended the original Directive: as a result, 12 newly identified substances were added (bringing the total to 45); the EQS of some existing substances was revised; and EQS in biota (plants and animals) were added for eight substances.

The challenge for the JDS3 was not only to review the occurrence of the priority substances which were found during previous surveys, but also to focus on the new priority substances and emerging pollutants which are not covered by legislation but are frequently detected in European rivers. Thanks to the cooperation of numerous European laboratories, the JDS3 became the largest scientific search ever on the Danube for unknown pollutants.

Notes on measurements
The **EQS** in water for priority substances are defined by the WFD for an average value of 12 measurements within one year, while the JDS3 only provided a single sample from August/September.

Hazardous substances are divided in this report into **heavy metals** and **organic compounds**.

2.1) Heavy Metals

Metallic elements, including those required for plant and animal nutrition, in trace concentrations which become toxic at higher concentrations.

Introduction
Heavy metals are elementary substances and natural parts of our environment, occurring in minerals, rocks and soils. As a result of widespread human use, metals are also present in industrial and municipal wastewaters, runoff from agricultural areas, and atmospheric deposition. If the tolerance levels in water and sediment are exceeded, then adverse effects in the aquatic ecosystem can result. Heavy metals can also limit drinking water supplies, affect livestock or disturb irrigation. Some metals can accumulate in the food chain leading to environmental or public health risks. During the JDS3, metal concentrations were measured in water, suspended particulate matter (SPM) and bottom sediment. The latter is of special interest, because metals can become remobilized if sediment is disturbed (e.g. through engineering or erosion).

WFD or not WFD?
Group 1: Heavy metals included in the WFD Priority Substances List: cadmium (Cd), lead (Pb), mercury (Hg) and nickel (Ni). Group 1 metals are regulated through WFD environmental quality standards (EQS).

Group 2: Other heavy metals assessed during the JDS2: arsenic (As), bismuth (Bi), cobalt (Co), chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo) and zinc (Zn). The JDS3 evaluation was done by comparing results with national regulation values.

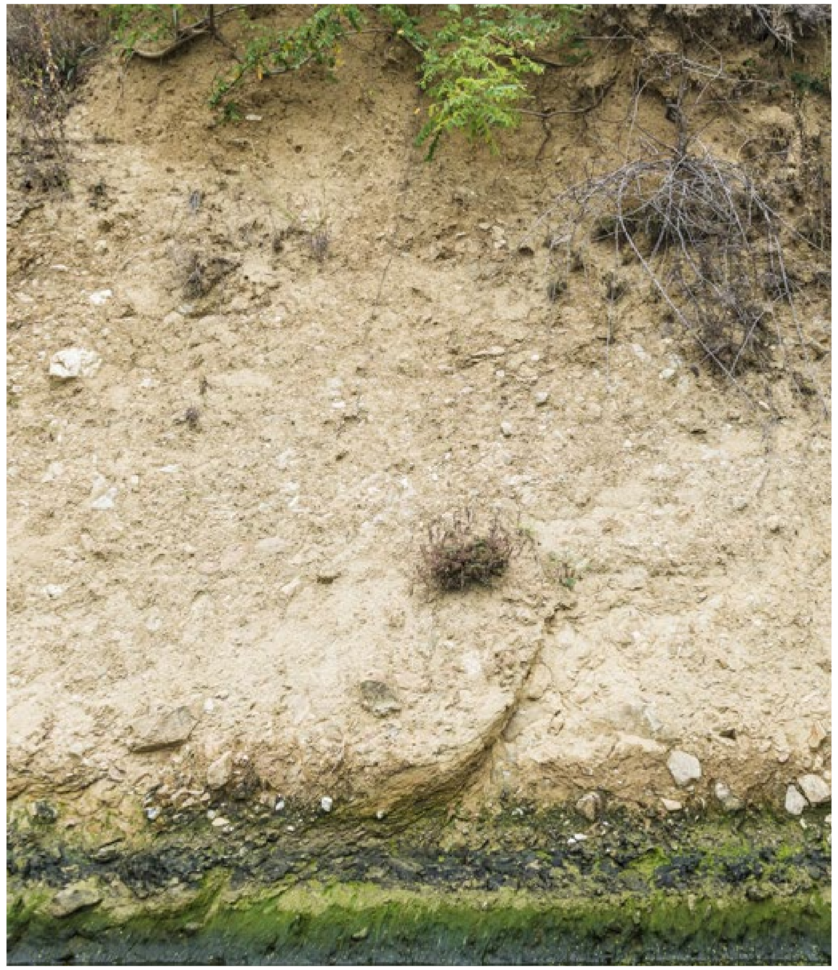
Results
In general, the concentrations of heavy metals and arsenic in water, and the contents of metals and metalloids in SPM and bottom sediments, were similar to those observed in the JDS1 and JDS2.

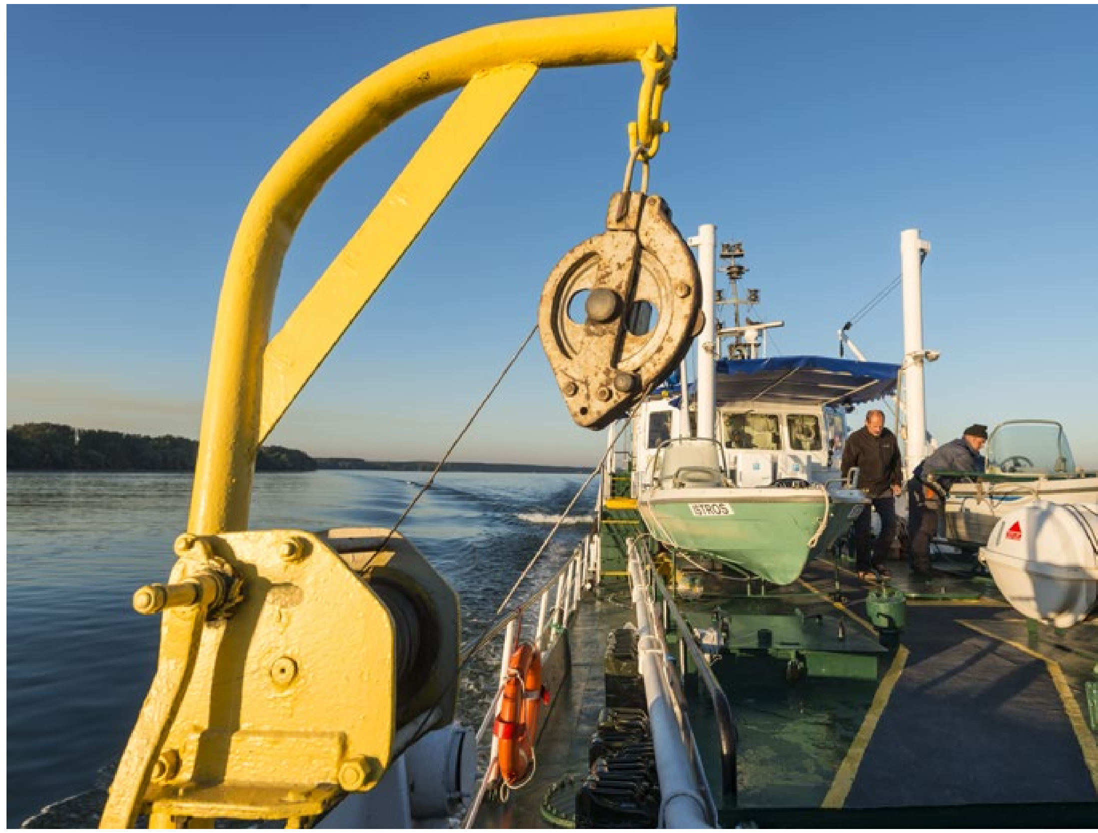
In water: No concentrations higher than the EQS were found for mercury or arsenic in water. EQS were exceeded for more than one element at only four Danube sites and one tributary: JDS9 Klosterneuburg (Ni, Pb, Cr); JDS11 Hainburg upstream Morava (Ni, Cr); JDS48 upstream Timok (Cd, Ni, Cu, Zn); JDS51 downstream Kozloduy (Ni, Cu); and the tributary Velika Morava JDS41 (Cd, Ni, Pb, Cr).

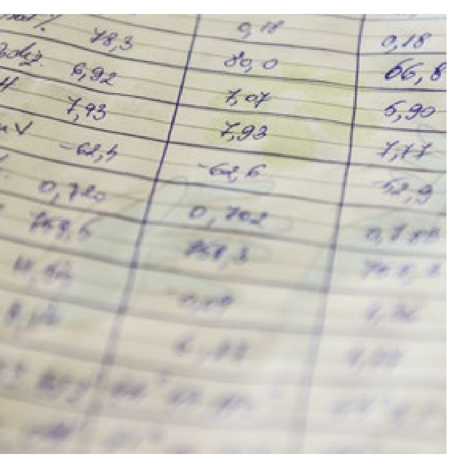
In SPM: JDS quality target values were exceeded as follows: As (1 site), Cu (3 sites), Ni (20 sites), and Zn (7 sites).

In sediment: Using German targets, only one exceedance was observed: Cu at JDS48.

In fish: Fish samples at six sites were tested for mercury by the EU's Joint Research Centre (JRC). The result was that all fish samples exceeded the EQS by factors of between five and 18. This exceedance has been reported by many other European countries, so the problem is not Danube-specific. Furthermore, the long-range transport of mercury from distant sources plays an important role, proven by exceedances that can be observed in pristine areas. Future results will show whether international efforts in reducing emissions will lead to a decline.







2.2) Organic compounds

In general, an organic compound is any member of a large class of chemical compounds whose molecules contain carbon.

Introduction

Organic pollution is caused mainly by wastewater from cities or towns, industry and agriculture, especially if the wastewater is untreated or inadequately treated. Organic pollution can cause significant impacts in water bodies, such as changing the level of oxygen and affecting populations of certain species.

Treating wastewater

Many cities and towns are neither connected to a sewage collecting system nor to a wastewater treatment plant. Across the Danube Basin, wastewaters are not collected at all in about 2,500 cities and towns (which have a population of at least 2,000 people). By the end of 2012, however, 555 urban wastewater treatment plants had been constructed, upgraded or extended which will help reduce organic pollution.

Note: The JDS3 analyzed many organic compounds, some of which are priority substances under the WFD, and some of which are not. While the next section focuses specifically on priority substances, the subsequent sections may or may not include priority substances, as explained.

2.2.1 Meeting the WFD: Priority substances

The selection of priority substances for the JDS3 analysis was based on: the present list of WFD priority pollutants; new environmental quality standards (EQS) for "old" WFD priority substances; "new" WFD priority substances; and results from the JDS1, JDS2 and ICPDR's Trans-National Monitoring Network (TNMN). Priority substances with known concentrations well below the current EQS (e.g. dichlorodiphenyltrichloroethane, or DDT) from other Danube surveys were not analysed. Priority pollutants were analysed in whole water, suspended particular matter (SPM), sediment and fish muscle. Some of the key findings are listed below:

DEHP has been used as a plasticiser worldwide in huge amounts for many years. During the JDS2, it was the most problematic priority pollutant, exceeding the EQS in 44% of water samples. However, during the JDS3, while DEHP in water was present in all samples, it was significantly below the EQS. DEHP was also found in higher concentrations in SPM and sediments during the JDS3, which shows that this pollutant is accumulating. Maximum concentrations in SPM and sediment samples were at JDS38 (upstream Pancevo/downstream Sava) and JDS9 (Klosterneuburg), respectively. Still, all concentrations were far below quality standards for the protection of bottom-dwelling organisms.

Perfluorooctansulfonic acid (PFOS) is a new priority substance under the WFD that repels water and oil and is resistant to heat and chemical stress. For the JDS3, PFOS exceeded the EQS at 94% of sampling sites.

Polar pesticides and biocides in water: As many pesticides and biocides are applied in April/July, the JDS3 sampling in August/September was not representative – therefore, only low concentrations were detected. Diuron, isoproturon and terbutryn (a "new" priority substance) were found to be below EQS, while studies showed the predominant use of terbutryn as a biocide.

C10-C13-chloroalkanes are widely used as plasticisers, additives in lubricants, cutting fluids and flame retardants. For the first time, C10-C13-chloroalkanes were analysed in water. All concentrations were found to be below the EQS.

Organotin compounds are used as biocides and in PVC manufacturing. Concerns over their toxicity have led to a range of restrictions in use, including EQS for tributyltin. During the JDS3, tributyltin was found at seven of 68 sites with values above the EQS: all sites with positive results were in the Upper/Middle Danube with the highest concentrations at JDS7 (upstream AbwindenAsten) and JDS12 (tributary Morava). Dibutyltin is also regulated in some countries, but no exceedances were observed during the JDS3. A comparison with the results of the JDS2 showed lower maximum values for tributyltin (and also dibutyltin) in 2013 – this reflects the effects of restrictions on this substance.

Hexabromocyclododecane (HBCDD) is a new priority substance. It is used as a flame retardant, mainly by the polymer and textile industry (e.g. in polystyrene insulation panels in building construction). For HBCDD, all samples showed values below the new EQS in biota.

AMPA, a degradation product of glyphosate (currently the most used herbicide worldwide but not regulated as a priority substance), was detected in most water samples with an unusual stable concentration level in all sections of the Danube. Concentrations up to five times higher in some tributaries had little influence on concentrations in the Danube.

Diclofenac, an important anti-inflammatory drug, is on the so-called 'Watch-List' for priority pollutants (a proposal designed to allow targeted EU-wide monitoring of substances of possible concern) with a proposed EQS. The only JDS3 sample to exceed this proposed EQS was from the Arges River.

2.2.2 Petroleum hydrocarbons

Among the organic pollutants, petroleum hydrocarbons (oil pollutants) are considered to be one of the most common and frequent. They are introduced from oil refineries, other industries, transportation, municipalities, and accidental releases.

Results

Total petroleum hydrocarbons (TPH) contamination in the SPM was lowest during the JDS1 and highest during the JDS2. TPH contamination in bottom sediment showed slowly increasing trends during the three surveys, probably due to increasing accumulation caused by the settling of contaminated SPM. The highest contamination levels were detected between the Gabcikovo and Iron Gate dams.

Polycyclic aromatic hydrocarbons (PAHs) occur in oil, coal and tar deposits as by-products of incomplete combustion processes. Some have been identified as carcinogenic, mutagenic and teratogenic. Eight substances were previously defined as priority pollutants. In water, the maximum concentrations of some PAHs showed that EQS were exceeded in some samples along the Danube: most of the highest values were found at site JDS24 (Dunafoldvar). For most of the PAHs in SPM, maximum concentrations were found at site JDS1 (BöfingerHalde). PAHs in sediment extracts showed a comparable level of contamination to the TPH, but even the highest concentrations were far below guidelines.

2.2.3 Dioxins and PCBs

Dioxins and dioxin-like compounds are new priority substances. Analyses were performed on polychlorinated dibenzo-p-dioxins and dibenzofurans **(PCDD/Fs)**, *indicator* polychlorinated biphenyl congeners (EC-6 **PCBs**), *dioxin-like* **PCBs** (DL-PCBs) and decabromodiphenylether **(BDE-209)** in selected samples of SPM and fish (*Abramis brama*) obtained from the JDS2 and JDS3.

PCDD/Fs are by-products of poor combustion and a variety of chemical processes. PCBs, and PBDEs such as BDE-209, are intentionally produced chemicals with many industrial and domestic applications such as dielectric fluids (e.g. used in transformers), paints, hydraulic oils, plasticisers, and flame retardants (compounds added to manufactured materials to prevent the spread of fire). PCDD/Fs and PCBs accumulate in the sediments and wildlife of aquatic systems. Although their production and emissions are strictly regulated in the EU, they still contaminate fish samples, often above the limits for food given by EU legislation, especially for the rivers Rhine and Elbe.

The toxic effects of PCDD/Fs and PCBs include dermal toxicity, immune toxicity, carcinogenicity, and adverse effects on reproduction, development, and endocrine functions. Although less evaluated, PBDEs are believed to have similar effects. The EU prohibited the use of BDE-209 in electronics and electrical equipment since July 2006.

Results

For PCDD/Fs and PCBs, none of the existing EQS values for aquatic biota and suspended solids/sediments, and none of the EU food limits concerned, were exceeded in the Danube.

2.2.4 Organophosphorus compounds (OPCs)

Organophosphorus compounds (OPCs) are commonly used as flame retardants in a variety of products, such as electronic equipment, plastics, rubbers, textiles and building materials. Since many brominated flame retardant (BFRs), including PBDEs, were banned in recent years, the use of OPCs as a substitute for PBDEs has increased. As a result, OPCs have been detected in many environments, such as effluent from sewage treatment plants. Chlorinated OPCs can pass through sewage treatment plants without being removed. OPCs are also carried through industrial wastewater discharge and long-range atmospheric transport. Some OPCs are bioaccumulative and can be found in freshwater and marine wildlife as well as in human breast milk. OPCs can be toxic to fish, daphnia and algae, and can have adverse human health effects (e.g. neurotoxic, carcinogenic or endocrine disruption). However, compared to PBDEs, the aquatic toxicities of OPCs are generally much lower.

Results

Generally, there are no local OPC emissions of concern for the Danube catchment. Among OPCs, TCPP clearly dominates in both the Danube and its tributaries **(figures 6 and 7).** The concentrations for all OPCs are several orders of magnitude below their toxic effect levels for aquatic wildlife.

2.2.5 Emerging substances

Emerging substances are chemicals discovered in water which have not been detected previously, or those detected at levels that may be very different than expected. They are not listed on the WFD list of priority substances. The risk of these substances to human health and the environment are often not known. They are usually not included in routine monitoring programs in major river basins and health or ecology guidelines have not yet been set. Many of the results below come from new techniques and strategies used during the JDS3.

Results

Pollutants with higher concentration levels were formylaminoantipyrine (FAA) and acetylaminoantipyrine (AAA) – metabolites (or products that remains after a drug is broken down by the body) of the drug metamizol, the artificial sweeteners acesulfame and sucralose, benzotriazoles (e.g. used to inhibit corrosion or in antifreeze), iodinated X-ray contrast media (substances involved in producing X-rays), and caffeine. Overall, concentration levels generally decreased slightly downstream the Danube to the Black Sea.

The concentrations for most of the contaminants were lower for the JDS3 compared to the JDS2. Carbamazepine, an antiepileptic drug, had slightly lower concentrations in the Lower Danube tributaries than observed during the JDS1 and JDS2. Caffeine concentrations in the Upper catchment were higher during the JDS3 while concentrations in tributaries were lower during the JDS3. Concentrations of AAA and FAA in the Danube appear to have increased.

Perfluorooctanoic acid (PFOA) was one of the most important contaminants in JDS2, coming mostly from a fluoropolymer production plant in Germany in the Inn River basin. PFOA concentrations were halved since the JDS2 as PFOA use in industrial applications was terminated at the end of 2008.

Figure 6: Summary of concentration ranges and average of OPCs in the Danube – dissolved phase

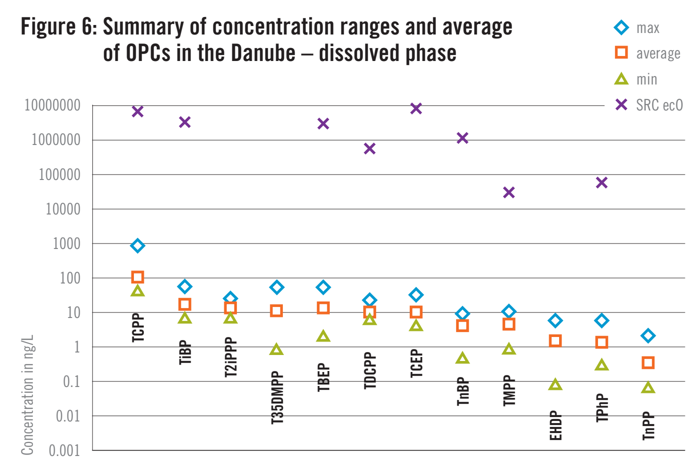
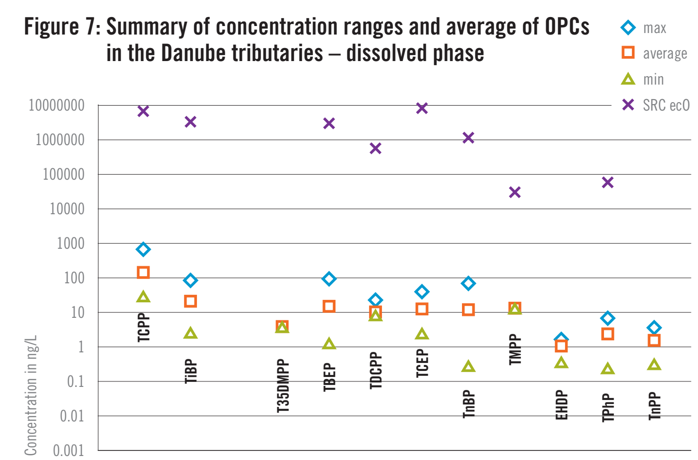


Figure 7: Summary of concentration ranges and average of OPCs in the Danube tributaries – dissolved phase



2.2.6 New JDS3 techniques and strategies

During the JDS3, many new techniques and strategies were used for the first time to analyze chemicals in the Danube Basin. These were meant to complement, not replace, more traditional water monitoring approaches that rely on WFD priority lists, river basin-specific compounds, or single-spot, single-moment measurements.

One of the goals was to detect many unknown or neglected organic compounds that are present in low concentrations. A typical water sample can contain some 10,000 chemicals from which scientists must separate the "good" from the "bad". Even though a chemical may be difficult to detect, it may still cause toxic effects. And when mixed with other toxins in and around the river, their potential adverse effects on the environment may also be unknown or unrecognized. In the end, many of the new JDS3 initiatives proved successful, and can serve as important tools for supporting the WFD and river basin management.

Effect-based screening

For highly diluted large rivers such as the Danube, effect-based screening requires the extraction of large volumes of water and its transport for laboratory analysis. For the JDS3, a newly developed, mobile, large-volume extraction device (LVSPE) was used to extract samples of up to 1000 litres at 22 sampling sites. Samples were then analysed for 264 organic compounds. **Bioassays** – scientific experiments involving the use of live animal or plant tissues or cells to measure the effects of a substance on a living organism – were also used.

Results: The compounds identified most often were pharmaceuticals, artificial sweeteners, corrosion inhibitors, and industrial chemicals. Widely used herbicides were also frequently detected.

Overall, low concentrations of organic compounds were found compared to other rivers in Europe. However, all extracts revealed, through bioassays, some degree of impact on one or more of the following: mutagenicity (can damage genes and cause cancer); drug metabolism (the body's conversion of drugs into other chemicals); oxidative stress responses (body's ability to detoxify or repair damage); estrogenicity (estrogen is a female hormone); and the inhibition of growth and photosynthesis of green algae.

These results show that attention should be given to the presence of organic compounds in the Danube beyond those listed in regulations.

Non-target screening

The goal of screening was to search for as many compounds as possible while focusing on previously unknown compounds.

Results: 110 out of 315 'searched for' substances were found in at least one sample, and 50 compounds were present in more than 20 samples **(figure 8, frequency of 110).** The substances 4-acetylaminoantipyrine, carbamazepine, 4-formylaminoantipyrine, DEET and 2,4-dinitrophenol were detected in all 68 site samples.

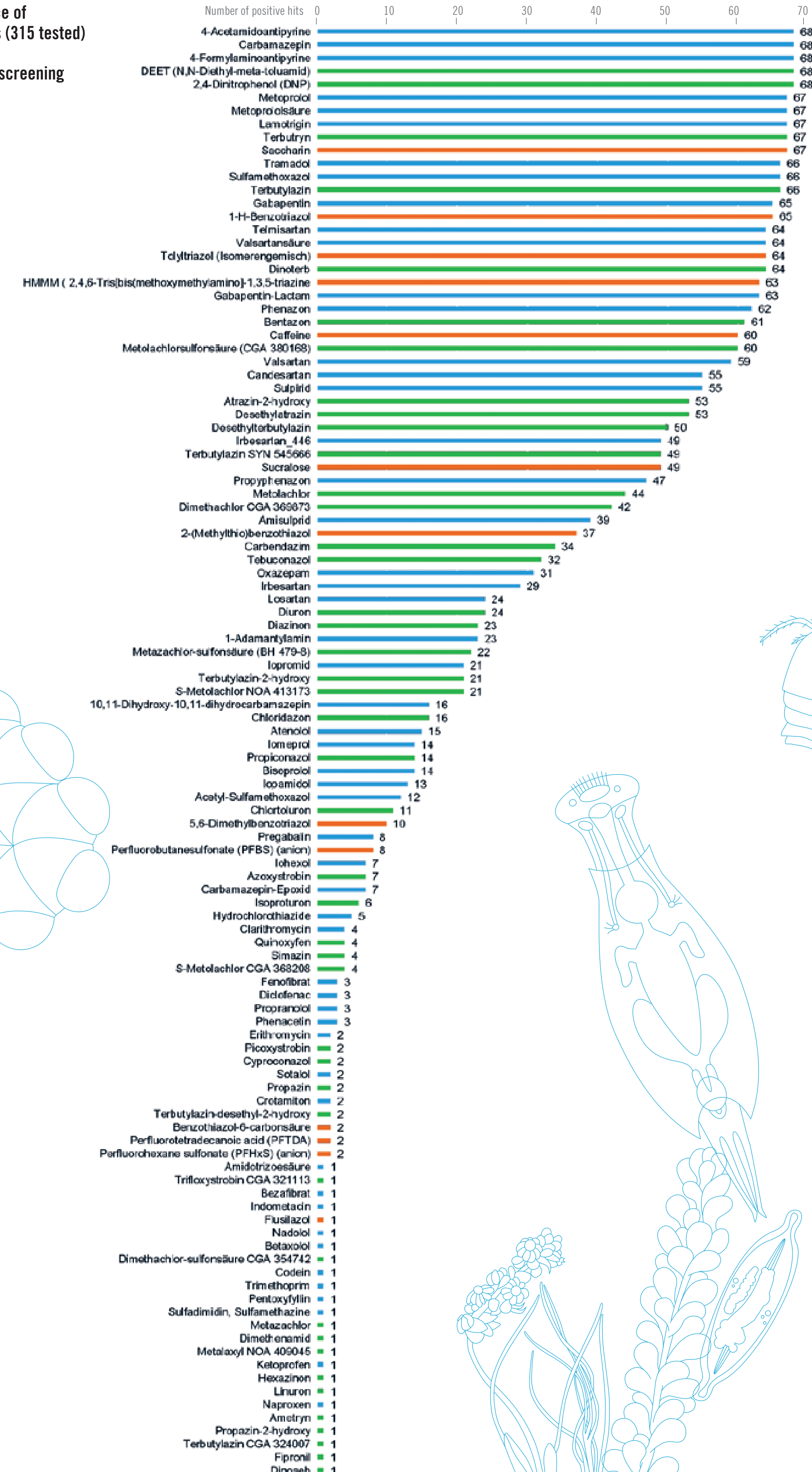
Passive sampling

Low-volume *spot sampling* of river water is conventionally used to detect organic pollutants. Spot sampling also shows the pollution at one individual JDS sampling site at a single moment of time. In contrast, *passive sampling* continuously samples pollutants for several days, including river stretches between individual JDS sampling sites. It can therefore detect small concentrations missed by spot sampling. It can also provide a picture of the pollution situation in different stretches of the Danube River and identify areas of concern for further investigation.

Results: Passive sampling helped to identify the concentrations of a broad range of organic pollutants in water, including PCBs, OCs, PAHs, alkylphenols, polar pesticides and pharmaceuticals. In most cases, passive sampling confirmed a similar distribution of pollutants along the river as was observed in the JDS2.

Figure 8: Frequency of appearance of 110 'identified' suspect pollutants (315 tested) in JDS3 surface water samples; results obtained from non-target screening workflow by HPLC-ESI-Q-TOF-MS operated in ESI+ and ESI− modes

Pharmaceutical drugs and metabolites
Pesticides and metabolites
Industrial chemicals and metabolites



Biomarkers

A *biomarker*, or biological marker, is a measurable indicator of a biological condition. It can detect the potential effects of a chemical on wild living populations. It can also show the biological impacts of specific river sites on species, even if other studies showed little or no interference.

Cellular DNA is continuously attacked by various agents (e.g. toxic chemicals) in the environment resulting in DNA lesions. Unrepaired DNA lesions can lead to mutations or cell death. To investigate, the JDS3 used the **'comet assay'** (biomarker) technique to detect DNA damage in cell nuclei, where detections appear as comet-like shapes. The research was performed on 217 specimens of mussels and 98 specimens of fish **(figure 9)**. Another assay, **'micronucleus frequency'**, evaluated the loss of chromosomes in fish cells.

Results: Using both the comet and micronucleus assays, significant variations in DNA damage levels were observed for different sampling sites for all selected species of mussels and fish.
For the comet assay, the highest levels of damage were observed in specimens collected in the Pannonian Plain Danube section, from rkm 1497-1071.

Checking the link between groundwater and surface water contamination

In many cities in the Danube Basin, drinking water comes from groundwater that is filtered naturally by river banks. However, pollutants can enter groundwater from the adjacent surface water, especially if they can dissolve in water and move easily in soil and groundwater. During the JDS3, for the first time, the link between the contamination of surface and ground water was observed – to help better understand the quality and cleaning capacity of these 'natural water treatment systems'. A set of 49 compounds was analysed.

Results: A number of emerging substances were detected in abstraction wells at bank filtration sites. This included amidotrizoic acid, iopamidol, acesulfame, benzotriazole and carbamazepine, which are known to last for a long time in the aquatic environment and are not completely removed by bank filtration. Concentrations in the abstraction wells were mostly low. An exception was the artificial sweetener acesulfame which was detected in most abstraction wells, although concentrations were not considered to be harmful for humans. However, acesulfame can act as an example of a persistent and mobile substance which is consumed in large quantities in food and drinks and which enters the water cycle through sewage water. Therefore, increasing pollution in the Danube and its tributaries with compounds having similar properties to acesulfame, especially when they are harmful, must be prevented.

2.2.7 Prioritization of specific pollutants

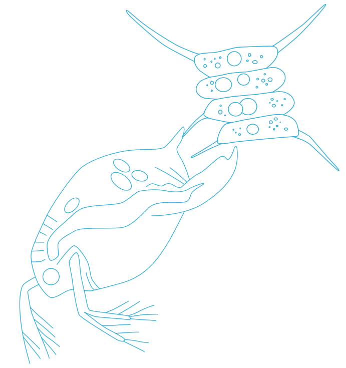
Given time and budget constraints, it is impossible to monitor and assess thousands of potential pollutants. So they should be prioritized. Under the WFD, Member States must set quality standards for "river basin-specific pollutants (RBSPs)" that are discharged in significant quantities, and take action to meet those standards by 2015. While most Danube countries have already defined their national RBSPs and related EQSs, there is no recent update of the *Danube River basin-wide list* of specific pollutants, which currently includes only arsenic, chromium, copper and zinc, without specifying their EQSs. Therefore, this study's aim was to prioritise among the large number of substances detected during the JDS3.

Results

A list of 22 substances relevant for the DRB was compiled, based on the results of the JDS3 target screening of 654 substances in Danube water samples by 13 laboratories. A substance was included only if it exceeded a threshold value (e.g. EQS) in at least one JDS3 site. 18 were found at more than 20 (out of 68) sites **(Table 1)**. The list contains five WFD priority substances (three PAHs, fluorathene and PFOS) and two candidate compounds on the EU Watch List (17beta-estradiol, diclofenac). The 'top ten' substances are dominated by (i) the pesticides 2,4- dinitrophenol (exceeding the limit value at all sites), chloroxuron, bromacil, dimefuron, and the transformation products of widely used atrazine and terbuthylazine, (ii) three polyfluorinated substances (PFOS, PFOA, PFNA) and (iii) the plasticiser bisphenol A, found at 30 sites.

More investigation is needed to determine whether these substances are true candidates for the Danube RBSPs. In addition, a separate prioritisation of hundreds of substances tentatively identified through non-target screening techniques will also be carried out. The ultimate goal is to pool all available data on organic pollutants in the DRB and prioritise them.

Figure 9: Representative micrographs of scored comets showing different levels of DNA damage (Tail intensity%)



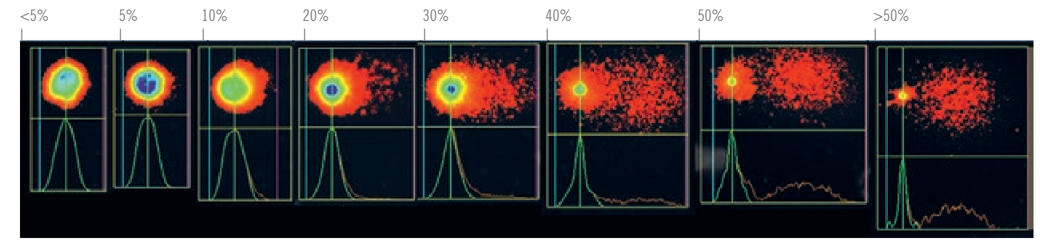
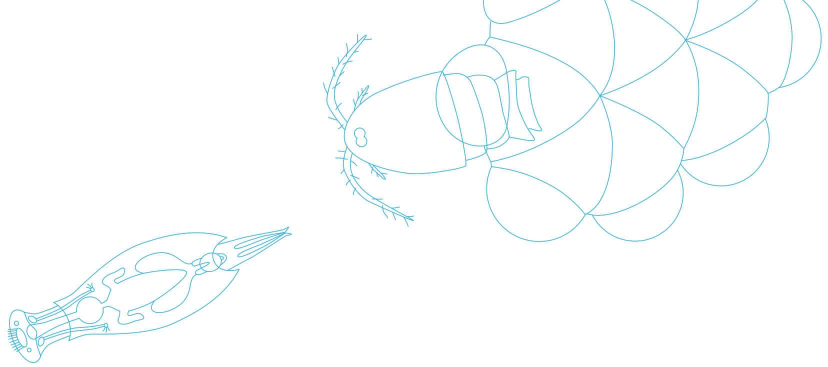


Table 1: Results of the prioritisation of pollutants determined in the JDS3 surface water samples

No.	Substance	CAS No.	No. of sites substance detected	C_{max}[1]	MEC_{95}[2]	Lowest PNEC/ EQS	Key study	Type	EoE[3]	EoE score	FoE[4]	Final score
1	2,4-Dinitrophenol (DNP)	51-28-5	68	0.06	0.04	0.001	RIVM 2014	EQS chronic water[5]	40	0.2	1.00	1.20
2	PFOS (Perfluorooctansulfonate)	1763-23-1	63	0.026	0.02	0.00065	EU 2013	EQS chronic water[5]	31	0.2	0.93	1.13
3	Chloroxuron	1982-47-4	65	0.04	0.02	0.0024	James et al. 2009	PNEC acute	8.3	0.1	0.93	1.03
4	Desethylterbutylazine	30125-63-4	54	0.028	0.01	0.0024	RIVM 2014	EQS chronic water[5]	4.2	0.1	0.79	0.89
5	2-hydroxy atrazine	2163-68-0	53	0.06	0.02	0.002	Ecostat 2013	EQS chronic water[5]	10	0.1	0.76	0.86
6	Bromacil	314-40-9	31	0.19	0.14	0.01	INERIS 2013	EQS chronic water[5]	14	0.2	0.46	0.66
7	Dimefuron	34205-21-5	58	0.041	0.04	0.008	Oekotoxzentrum 2014	EQS chronic water[5]	5.0	0.1	0.56	0.66
8	Bisphenol A	80-05-7	30	1.94	1.03	0.1	Nendza 2003	EQS chronic water[5]	10	0.2	0.16	0.36
9	Benzo(g,h,i)perylene	191-24-2	65	0.029	0.003	0.002	CEC 2008	EQS chronic water[5]	1.5	0.1	0.26	0.36
10	Diazinon	333-41-5	21	0.009	0.01	0.001	Management Team PPDB 2009	PNEC acute	10	0.1	0.12	0.22
11	Indeno(1,2,3-c,d)pyrene	193-39-5	15	0.005		0.002	CEC 2008	EQS chronic water[5]			0.19	0.19
12	Linuron	330-55-2	32	1.42	1.12	0.26	Oekotoxzentrum 2014	EQS chronic water[5]	4.3	0.1	0.07	0.17
13	Amoxicillin	26787-78-0	33	0.28	0.08	0.078	van der Aa et al. 2011	PNEC chronic	1.0	0.1	0.03	0.13
14	Metazachlor	67129-08-2	30	0.03	0.02	0.019	INERIS 2014	EQS chronic water[5]	1.1	0.1	0.03	0.13
15	17beta-estradiol	50-28-2	8	0.029		0.0004	CEC 2011	EQS chronic water[5]			0.12	0.12
16	Benzo(a)pyrene	50-32-8	3	0.002		0.00017	EU 2013	EQS chronic water[5]			0.04	0.04
17	Diclofenac	15307-79-6	51	0.318	0.036	0.05	Oekotoxzentrum 2014	EQS chronic water[5]			0.04	0.04
18	Bentazon	25057-89-0	61	0.1	0.02	0.06	USEPA 2008	PNEC acute			0.01	0.01
19	Fipronil	120068-37-3	1	0.02		0.012	EU 2011	EQS chronic water[5]			0.01	0.01
20	Fluoranthene	206-44-0	58	0.02	0.006	0.0063	EU 2013	EQS chronic water[5]			0.01	0.01

1 C_{max} – Maximum concentration in µg/L reported in case the substance has been measured by several JDS3 laboratories
2 MEC^{95} – 95^{th} percentile of the Maximum Environmental Concentration in µg/L; calculated only if the substance has been found above LOQ at minimum 20 sites
3 EoE – Extent of Exceedance
4 FoE – Frequency of Exceedance
5 Equal to Annual Average EQS (AA-EQS)

Hydromorphology Assessment



Hydromorphology: The physical characteristics of a water body's shape, structure or natural flow.

Introduction

'Good hydromorphology' is essential for meeting the requirements of the WFD because it contributes to 'good ecological status' whereby waters must provide good conditions, such as migration routes and suitable habitats, for natural species to live healthily.

In the Danube Basin, alterations to hydromorphology are caused mainly by hydropower generation, navigation and flood protection. For example, some 80% of the Danube basin's former wetlands and floodplains are disconnected, largely due to the past expansion of agricultural uses and river engineering works for flood control, navigation and power generation. 304 water bodies in the basin are affected by barriers for fish migration: in response, the re-establishment of free migration routes has become a key goal.

Fish and the Iron Gates dams

The Iron Gate dams I & II at the border between Romania and Serbia are a specific challenge. They represent the first impassable obstacles for fish migration along the Danube River from the Black Sea. Restoration here would re-open a reach of more than 800 km, providing access to habitats and spawning grounds along the Danube and its tributaries for sturgeons and other migratory fish species.

Methods

Two different approaches were used to assess hydromorphology. The first, the 'continuous survey', assessed the entire 2,415 rkm of the Danube River, subdividing it into 241 segments of 10 rkm lengths plus 18 segments for branches in the Danube Delta. All of the data was obtained by using high-resolution image analysis, maps and field observations. This approach used WFD parameters for morphology (e.g. river depth), hydrology (e.g. quantity of water flow) and river continuity (e.g. impacts of dams). The five classes of assessment were: 1 – near natural, 2 – slightly modified, 3 – moderately modified, 4 – extensively modified, and 5 – severely modified. The assessment was further organized according to the main Danube channel, banks and floodplains, as well as the three Danube reaches.

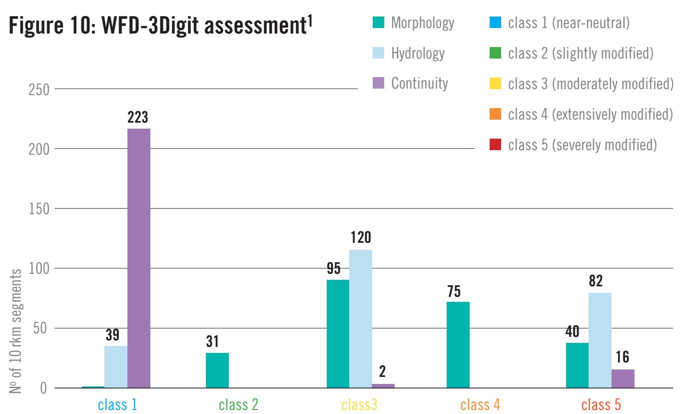
The second approach, performed for the first time on the Danube, consisted of detailed individual site analyses for each of the 68 JDS3 sampling sites. This was especially useful to support the biological assessment under the WFD, for example, to provide detailed physical habitat data (e.g. for fish).

Results (see figures 10 and 11)

Overall: About 60% of the Danube stretch falls below class 3, with 21% in class 2 (slightly modified) and 39% in class 3 (moderately modified). However, 40% falls in the two worst classes: class four (26%), and class five (14%). The overall picture is therefore split into one large part with satisfactory conditions and a second part with totally altered reaches. The "poor" assessment in the Upper Danube differs significantly from the comparatively "good" assessment in the Lower. The assessment also confirms the main findings of the JDS2.

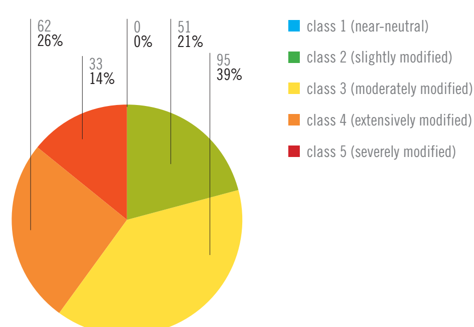
Danube channel: Many segments fall under classes 2 and 3, especially for the long free-flowing stretches in the Middle and Lower Danube. About 590 km fall in the worst class because of impoundments and severely altered stretches within dense settlements.

Figure 10: WFD-3Digit assessment[1]



1) For "Hydrology" and "Continuity" only the classes 1, 3 and 5 were evaluated

Figure 11: CEN-Overall assessment (with colour and assessment schema)



Banks: Over 25% of the surveyed banks fall into classes 1 and 2, mainly in the Lower Danube. Many fortified banks, belonging to classes 4 and 5, can be found along the Upper Danube, where higher degrees of urbanisation and hydropower also cause negative impacts.

Floodplains: Very few stretches still host good conditions and space for floodplains. Floodplains have been lost in at least 65%-70% of the river represented by classes 4 and 5 and partially by class 3. Floodplains that do remain often suffer from disconnection with the river, sediment build-up from dams, and poplar plantations substituting for natural floodplain vegetation.

Upper Danube: Some 75% is intensively changed, with many segments affected by impoundments and intensive river regulation works. Only the still free-flowing reaches between Straubing and Vilsofen in Bavaria as well as Wachau and the Vienna-Morava confluence fall into the "moderate" class, representing about 25% of the total.

Middle Danube: About 13% of the Middle Danube has good hydromorphological conditions while nearly 50% falls in the moderate class. The remaining 37% in classes 4 and 5 can be found in the two reservoirs of the Gabcikovo and Iron Gate I dams (the only dams in this reach) as well as the city reaches of Budapest and Belgrade.

Lower Danube: Over 40% of the Lower Danube stretch falls into class 2, which is remarkable in comparison with the Upper Danube or the Lower Rhine River. The Lower Danube has the longest free-flowing stretch of the Danube, with 860 km. Moderate class is found in about 40%, including "town and harbour" stretches and free-flowing stretches with moderate regulation works and/or cut floodplains. The remainder, 19% in classes 4 and 5, is in the Iron Gate II segment (Iron Gate II is the only dam in this reach) and canalised Sulina channel in the Delta. However, the entire Lower Danube is heavily influenced by the Iron Gate dams and some major tributaries.

Recommendations

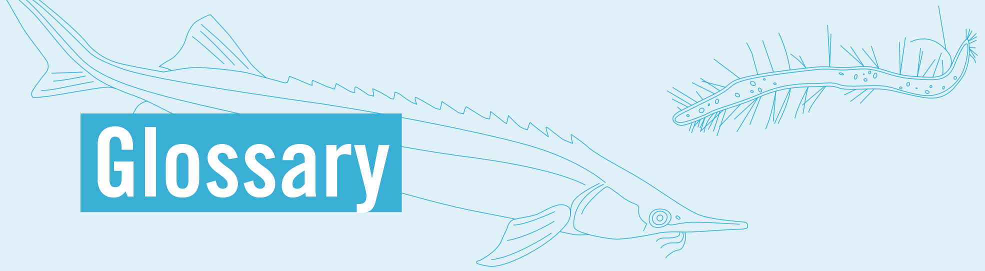
Sustainable restoration actions should be continued to help meet the good ecological status/potential along the entire Danube. Floodplain restoration should be a long-term goal for ecological and flood mitigation planning. The impacts of existing dams should be a matter of further basin-wide investigations. And given the fact that many large European rivers are severely altered, less altered water bodies along the Danube should be carefully managed.

Connecting birds with healthy river processes

In the spring of 2011 and 2013, the Danube River Network of Protected Areas monitored two birds as indicators for assessing hydromorphology. The first, the Little-Ringer Plover, breeds on sand and gravel banks or islands. The second, Sand Martins, have breeding holes in steep river slopes washed out by water. The birds depend on these habitats, and the habitats depend on river dynamics such as rising and falling water levels and erosion processes. Take away the dynamics, however, and many habitats and species become rare – those on the Danube have experienced high rates of extinction!

The 2013 survey, made in conjunction with the JDS3, had 56 experts from 13 Danube Protected Areas assess 4,119 km from small boats. The results showed high natural value for the Middle and Lower Danube and many tributaries. In the Upper Danube, the absence of Sand Martin and low density of Little Ringed Plover indicated heavy hydromorphological alterations, although the high number of territories of Little Ringed Plover on the last remaining free-flowing sections indicated the high potential of river restoration projects. For Plover, an extraordinarily high density was recorded for the Drava River. The highest densities of Sand Martin were recorded along the Sava and Drava rivers. Research showed that Plover clearly prefer island-like structures. The study also showed that, in river sections in class 3 (moderately modified) and class 4 (extensively modified), the probability of occurrence of one of the two species was reduced to about 65% and 30%, respectively.

Glossary



Abundance (species): The number of individuals per species.

Assay: A procedure measuring the presence or amount or the functional activity of a target entity.

Atmospheric deposition: Chemicals or other substances that are deposited from the atmosphere onto the surface (e.g. land, water).

Benthic: Bottom of a sea or lake.

Bioaccumulation: The accumulation of substances, such as pesticides, or other organic chemicals in an organism.

Bioassay: Involves the use of live animal or plant or tissue or cell to determine the biological activity of a substance.

Biocide: A biocide is a chemical substance or microorganism which can deter, render harmless, or exert a controlling effect on any harmful organism by chemical or biological means.

Biodiversity: The variation of life forms within a given ecosystem, biome or for the entire Earth. Biodiversity is often used as a measure of the health of biological systems.

Biomarker: A measurable indicator of a biological condition.

Biomass: Biological material derived from living or recently living organisms.

Biota: Plants and animals.

Carcinogenic: Capable of causing cancer.

Chlorophyll-a: A green pigment found in plants and cyanobacteria.

Composition (species): The identity of all the different organisms that make up an ecological community.

Confluence: The meeting of two or more bodies of water.

Crustacean: This large group of species includes various familiar animals such as crabs, lobsters, crayfish, shrimp and barnacles. The majority of them are aquatic.

Cyanobacteria: A type of bacteria that obtains its energy through photosynthesis (*cyano* means blue).

Danube River Basin Management Plan: The WFD requires all EU countries to have River Basin Management Plans, including a Programme of Measures, by 2009 and to update them in 2015 and 2021. The DRBM Plan Part A (Basin-wide overview) is coordinated by the ICPDR and based on the national RBM Plans.

Danube River Protection Convention: Signed in 1994 by Danube countries and the EU, it is the major legal instrument for cooperation and transboundary water management in the Danube River Basin.

Density (species): The number of individuals of a species in an area.

Diatoms: A major algae group and one of the most common types of phytoplankton.

Diversity (species): The number of species within a biological community (also known as "richness").

DNA: Deoxyribonucleic acid is a molecule that encodes the genetic instructions used in the development and functioning of all known living organisms and many viruses.

Dominance (species): The species that predominates in an ecological community, particularly when they are most numerous or form the bulk of the biomass.

Electric fishing: The act of using an electric field in water to stun fish so they can be collected with a net, assessed and then released, usually unharmed.

Emerging substances: Chemicals discovered in water which have not been detected previously, or those detected at levels that may be significantly different than expected.

Endocrine disrupting compounds: Organic compounds which can significantly impact the hormones of animals such as humans, fish and snails.

Environmental quality standards (EQS): Under the WFD, EQS refer to commonly agreed concentration levels that are acceptable for "good chemical status", used by scientists as toxicity indicators.

Eutrophication: Elevated production of biomass in waters mainly due to an overload of nutrients (typically nitrogen or phosphorus).

EU Watch List: A proposal designed to allow targeted EU-wide monitoring of substances of possible concern.

Faeces: Excrement; or waste expelled from an animal's digestive tract.

Fauna: A typical collection of animals found in a specific time or place.

Fish: Aquatic vertebrates (having a backbone) that are typically cold-blooded and covered with scales.

Flame retardant: Compounds added to manufactured materials to prevent the spread of fire.

Floodplain: Any land area susceptible to being inundated by floodwaters from any source.

Flora: A typical collection of plants found in a specific time or place.

Food chain (or web): Shows how organisms are related with each other by the food they eat.

Good biological and ecological status: The quality required for a water body to meet WFD requirements.

Habitat: The physical and biological environment on which a given species depends for its survival.

Helophytes: Plants that grows in a marsh, partly submerged in water.

Hydromorphology: As defined by the WFD, the physical characteristics of the shape, boundaries and content of a water body.

Hydrophytes: Free-floating or submerged plants.

Immunotoxicity: Toxicity to the immune system.

Impoundment: A reservoir formed by a dam.

International Commission for the Protection of the Danube River (ICPDR): The international organisation which has been established to implement the Danube River Protection Convention.

Invasive species: Non-indigenous species (e.g. plants or animals) that adversely affect the habitats they invade economically, environmentally or ecologically.

JDS1: The first Joint Danube Survey coordinated by the ICPDR in 2001.

JDS2: The second Joint Danube Survey coordinated by the ICPDR in 2007.

JDS3: The third Joint Danube Survey coordinated by the ICPDR in 2013.

JDS x,y...: JDS sampling site numbers

Joint Program of Measures: Part of the DRBM Plan Part, this is a summary of the national Programmes of Measures and some of the common activities of the Danube Basin countries in the ICPDR.

Macroinvertebrates: Aquatic insects, worms, clams, snails and other animals without backbones that can be determined without the aid of a microscope and that live in or on sediments.

Macrophytes: Aquatic plants, either free-floating or attached to the bottom, which can be determined by the naked eye without the need for a microscope.

Metabolism: Includes all the things your body does to turn food into energy and keep you going.

Microbiology: The study of microscopic organisms that are unicellular or exist in cell clusters.

Mutagenic: Can damage genes and possibly cause cancer.

Nutrient: Substances such as nitrogen and phosphorus, used by organisms to grow.

Nutrient pollution: Contamination of water resources by excessive inputs of nutrients. In surface waters, excess algal production is a major concern.

Nutrient retention: Plants are able to absorb and retain nutrients, and therefore reduce nutrient pollution.

Order of magnitude: An amount equal to ten times a given value.

Organic compounds: Natural or synthetic substances based on carbon.

Organic pollution: Occurs when an excess of organic matter, such as manure or sewage, enters the water.

Parameter: A characteristic, feature, or measurable factor that can help in defining a particular system.

Pathogens: Bacteria, viruses, parasites or fungi that can cause disease.

Pelagic: Any water in a sea or lake that is neither close to the bottom nor near the shore.

Perfluorinated acids: Chemicals that repel water and oil and are resistant to heat and chemical stress.

Persistent organic pollutants (POPs): Chemicals that remain intact in the environment for long periods, become widely distributed geographically, accumulate in the fatty tissue of living organisms and are toxic to humans and wildlife.

Pesticide: A substance, usually chemical, used to kill unwanted plants and animals.

Phytobenthos: Microscopic plants such as algae that live in the bottom layers of the river and seabed.

Phytoplankton: Plants, mainly microscopic, existing in water bodies.

Point source: A well defined source of pollution from a single point, such as a pipe. **Non-point sources** of pollution enter water from a dispersed (or "diffuse") and uncontrolled source, such as runoff from land or from the atmosphere, rather than through a pipe.

Plasticiser: Substances that increase the plasticity or fluidity of a material, especially for plastics such as polyvinyl chloride (PVC).

Ponto-Caspian origin: From the Black, Caspian or Azov Sea.

Primary producers: Use sunlight, water, chlorophyll and carbon dioxide to synthesize organic compounds.

Priority substances: The EU's 'Priority Substances' or groups of substances which have been shown to be of major concern for European waters. Priority Substances include organic compounds and heavy metals.

Radioactivity: The spontaneous discharge of radiation from atomic nuclei.

Reach: The Danube is split into three "reaches" (see page 9 box for more).

Rip-rap: Large boulders that have been artificially placed to fix riverbanks, especially at channelized and impounded river sections.

Rkm: Distance in the river upstream from the river's mouth (for the Danube River, distance from the Danube Delta).

Sediment: Material that was suspended in water and that settles at the bottom of a body of water.

Species abundance: The number of individuals per species. **Relative abundance species** is the species abundance relative to the abundances of other species represented in the community.

Species diversity: The number of species within a biological community (also known as "species richness").

Substrate: The surface on which a plant lives.

Suspended sediment refers to the solid particles, suspended within the water column, which the water is carrying. Also known as **suspended particulate matter (SPM).**

Taxon (sg), Taxa (pl): A group or category of living organisms.

Teratogenic: Capable of causing birth defects.

Toxicity: The degree to which a substance can damage an organism.

Toxicology: Study of the effects of chemicals on living organisms.

Trans-National Monitoring Network (TNMN): Coordinated by the ICPDR, it comprises over 75 monitoring stations and provides a regular overview of the main chemical and physical parameters important for assessing water quality.

Tributary: A river that flows into a larger river or other body of water.

Turbidity: The cloudiness or haziness of a fluid caused by large numbers of individual particles that are generally invisible to the naked eye.

Vascular plants: Having tissues for conducting water and minerals throughout the plant.

Water Framework Directive (WFD): Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy.

Zooplankton: Tiny invertebrates (animals without backbones) that float freely in water bodies.

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A substantial part of the laboratory services for the JDS3 were provided as free contributions by the ICPDR's member countries. Leading national hydro-analytical laboratories from Germany, Austria, the Czech Republic, Slovakia, Croatia and the European Commission's Joint Research Centre in Ispra, Italy, carried out chemical analyses.

Laboratory biological analyses were performed by the Core Team members and their respective institutions after the expedition. Additional contributions came from TZW Karlsruhe (Germany), BOKU Vienna (Austria), the Medical University in Vienna, and from the partner laboratories of the NORMAN network.

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