# INTERNATIONAL COMMISSION FOR THE PROTECTION OF THE DANUBE RIVER

# Joint Danube Survey: Investigation of the Tisza River and its tributaries

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Prepared by

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# 1 Preface

The Investigation of the Tisza River (ITR) project was an international expedition carried out on the Tisza River by a long-sectional sampling using a German Laboratory ship, ARGUS. The program directly followed the Joint Danube Survey (JDS). The mission was organised by the Secretariat of the International Commission for the Protection of the Danube River (ICPDR) in Vienna. The Hessian Agency for the Environment and Geology (Wiesbaden, Germany) provided the Laboratory Ship to perform the ITR program. It was a follow-up activity to the pollution caused by cyanide and heavy metal from accidents that happened in February and March 2000 on the Szamos and Tisza Rivers. One of the objectives of the survey was to detect the present environmental situation on the River Tisza and its main tributaries one and a half year after these accidents.

Similarly to the JDS mission, an international team of experts formed the Core Team of scientists during the ITR expedition. In addition to that several National Teams were formed from the experts of the Regional Environmental Inspectorates that joined the ITR expedition at the particular Tisza sections.

The Study was financially supported by the European Commission. Essential in-kind contribution was received from the Government of Germany. The Tisza riparian countries (Romania, Slovakia, Yugoslavia and Hungary) also provided important support to the expedition. The Tisza Survey was organized by the Secretariat of the ICPDR (Igor LISKA, Project Coordinator, Jaroslav SLOBODNIK, Logistics Officer) providing help to the overall organization and financial planning. The Water Resources Research Center Plc. (VITUKI Rt.). Budapest, Hungary assured technical coordination.

The sampling mission was carried out between 28 September and 10 October 2001. The samples were analyzed in Hydrometeorological Institute, Belgrade, in the VUVH, Bratislava, and in the VITUKI, Budapest. Béla CSÁNYI (Project Manager) and Ferenc LÁSZLÓ (Director, Institute for Water Pollution Control, VITUKI) prepared the ITR report with the contributions of Petra Stahlschmidt-Allner (Germany), Carmen Hamchevici (Romania), Jarmila Makovinská (Slovak Rebublic), Jovanka Ignjatovic (Federal Rebublic of Yugoslavia). The report was reviewed by the Monitoring, Laboratory and Information Management expert group of the ICPDR. The findings, interpretations and conclusions of the ITR Study are available in the ICPDR web site as part of the JDS international sampling program itself.

# 2 Background and objectives

Several (acute and chronic) accident pollution happened in the Tisza River during the year of 2000 due to industrial activities in the upper (Ukrainian and Romanian) watershed. Reliable and comparable information on chemical and biological status is necessary in order to describe the effect of the pollution events, and to compare the present pollution level to any future post-pollution situation.

German and Hungarian scientists agreed to perform the "Investigation of the Tisza River (ITR)" to study the environmental effects of the acute cyanide pollution and frequent chronic heavy metal pollution accidentally occurring in the Tisza River Basin. The ITR program followed directly the Joint Danube Survey (JDS) organised by the MLIM EG of the International Commission for the Protection of the Danube River (ICPDR) after it was finished in the Danube Delta on the 20th of September 2001. The overall objective of the ITR was to undertake a longitudinal ship survey of the Tisza River to produce comparable and reliable information on the quality of different compartments of the aqueous ecosystem (sediment, suspended solids, mussel, macroinvertebrate and fish species) with focus on chemical and biological determinands.

Earlier national monitoring program was performed in Hungary between 2000 and 2002 sponsored by the Hungarian Ministry of Environment. The purpose of the study was to describe the acute effect of the cyanide and heavy metal pollutions together with the investigation of further effects of the polluting compounds on the aquatic environment. Seasonal investigations were performed on the macroinvertebrates and detailed analysis was done on the heavy metal pollution of the sediment fraction after the main Tisza flooding period of the Tisza in April 2000. Data collected during that period are ready for comparison of the present ITR in order to describe the environmental effects on this short time scale.

VITUKI Plc, Budapest, Hungary in co-operation with the Hydrometeorological Institute, Belgrade, Federal Republic of Yugoslavia, performed a special sampling program "Study on Bioindicators, Inorganic and Organic Micropollutants in Selected Bioindicator Organism in the River Danube" during the summer period of 2000. It was a follow-up activity after the UNEP/OCHA Balkan Task Force Mission, which investigated the environmental impacts of the Kosovo conflict in FRY in 1999. The major aim of the Study on Bioindicators was to investigate the presence and accumulation of organic and inorganic micropollutants in sediments and biota and to analyse the macrozoobenthos in the Danube reach and its main tributaries impacted by the Kosovo conflict. The Study was financially supported by the governments of Germany and Austria and organized by the Secretariat of the ICPDR. The collected data on micropollutant compounds are available for comparison in case of the ITR results, as well.

The intention of the JDS - ITR project was to use the *same sampling and laboratory expertise* that had been applied for the JDS expedition, and, in the Danube countries, as well. Additionally, it seemed to be useful to use the *same resources* that have the necessary level of analytical instrumentation and operate with proven acceptable AQC procedures. Therefore the goals of the ITR survey were as same as for the JDS:

- To produce a homogenous data set for the Tisza River based on the analysis of specified determinant(s).
- To provide a forum for participation of riparian/river basin country in sampling and inter-comparison exercises (German, Romanian, Slovakian, Yugoslavian and Hungarian experts).
- To facilitate specific training needs and improve in-country experience.
- To promote public awareness.
- To indicate the special attention of the European Community to the environmental issues of East-European transboundary rivers

The documentation of the survey activities concerning the sampling and analytical techniques will serve as a training material for widespread distribution in Danube countries. The final reports of the JDS and the ITR would like to help in strengthening the public awareness within the ICPDR countries.

# **3 Preparation for the ITR survey**

### 3.1 Sampling plan and sites

Similarly to the sampling plan of the JDS mission, on board analysis of water, sampling the sediment and the suspended solids, and, different hydrobiological investigations were carried out in the same time during the ITR program. The sampling of phyto- and zooplankton together with the macroinvertebrate community were included during the mission. Parallel to the program of ARGUS, a fish-biological group from Germany was working with the help of a Hungarian expert to sample different fish species in order to carry out physiological studies concerning the possible effects of endocrine disrupters on fish.

According to the approved timetable of the ITR, the Core Team members and the ARGUS met in Belgrade on a press conference on 28 September, exactly one week later than the JDS mission was finished in Tulcea. The first sampling cross section was reached on 29<sup>th</sup> in Titel, 2 kms upstream from the Danubian confluence. Due to the rainy weather few weeks earlier, there were flooding conditions in the Tisza and its tributaries, especially in the Maros River during the sampling mission.

Altogether 27 sampling sites were included in the ITR program, 20 of which were situated in the Tisza River, and 7 in the main tributaries. Four sites were included in the Yugoslavian stretch. *Table 3.1* indicates the sampling locations and *Figure 5.1* illustrates their spatial distribution in the map.

The Tisza is navigable at normal (middle) water level up to Vásárosnamény-Gergelyiugornya. Due to the fast decrease of water level during the final stage of the ITR program, the upper four Tisza section and the Szamos were sampled by car in order to avoid shipping problems.

Station No.	Country	Town/Location or Tributary Name	Km-index	Date of sampling
ITR 1	YU	Titel, Danubian confluence	2	2001.09.29
ITR 2	YU	Bega Navigable Canal	3	2001.09.29
ITR 3	YU	Novi Becej	66	2001.09.29
ITR 4	YU	Novi Knezevac	147	2001.09.30
ITR 5	YU, H	Szeged, old city bridge	174	2001.10.01
ITR 6	Н	Maros, Makó	172	2001.10.01
ITR 7	Н	Mindszent	216	2001.10.02
ITR 8	Н	Hármas-Körös, Kunszentmárton	234	2001.10.02
ITR 9	Н	Tiszaug, híd	266	2001.10.03
ITR 10	Н	Szolnok	334	2001.10.03
ITR 11	Н	Zagyva, Szolnok	335,6	2001.10.03
ITR 12	Н	Kisköre	404	2001.10.04
ITR 13	Н	Aranyosi-sziget (Kisköre Reservoir)	419	2001.10.04
ITR 14	Н	Tiszafüred-Poroszló	431	2001.10.04
ITR 15	Н	Tiszacsege	453	2001.10.05
ITR 16	Н	Polgár	498	2001.10.05
ITR 17	Н	Sajó, Kesznyéten	492	2001.10.05
ITR 18	Н	Rakamaz-Tokaj	544	2001.10.06
ITR 19	Н	Bodrog, Bodrogkeresztúr	544	2001.10.06
ITR 20	Н	Tiszabercel	569	2001.10.07
ITR 21	Н	Dombrád	592	2001.10.07
ITR 22	Н	Tuzsér	616	2001.10.07
ITR 23	Н	Aranyosapáti	669	2001.10.08
ITR 24	H	Gergelyiugornya	685	2001.10.08
ITR 25	H	Szamos, Tunyogmatolcs	686	2001.10.08
ITR 26	H	Tivadar	705	2001.10.08
ITR 27	H	Tiszabecs	744	2001.10.08

# Table 3.1 Sampling stations along the Tisza River and its tributaries during theJDS Investigation of the Tisza (ITR) international sampling mission

Colour indication:

ITR No. X Sampling sites on the Tisza River ITR No. Y Sampling sites on the tributaries

Note that river km-s of tributaries indicate the position of cross section of confluence instead of the real sampling location. Sampling site of the cross section in tributaries usually situated 2 km upstream the confluence.



# 3.2 Arrangements for the survey

# 3.2.1 Core Team

The main contractor (VITUKI Rt.) provided four persons to the Core Team. One scientist from Romania, Slovakia and Yugoslavia were included also in the Core Team from the beginning of the ITR program. In the German fish-biological team two Core Team members and five joining colleagues followed the ARGUS ship by car between 29 September and 5 October carrying out local fishing activity with an Hungarian ichthyologist. They met the ARGUS team in Tokaj and since then they were dealing with the sample processing activity on the board.

The names of scientists in the Core Team and in the joining group are as follows.

Position	Name	Responsibility
JDS ITR Core Team Leader	Bela Csanyi	Sampling suspended solids and fractionation of sediments; dredging, sampling zooplankton, macrozoobenthos and mussels
Chemist	Carmen Hamchevici	On-board determinations of nutrients.
Biologist	Momir Paunovic	Grabbing, sampling macrozoobenthos, on-board selection.
	Jarmila Makovinska	On-board determinations of chlorophyll-a and sampling of phytoplankton.
	Peter Juhasz	Dredging, sampling macrozoobenthos and mussels, on- board selection.
	Viktoria Kavran	Grabbing, sampling macrozoobenthos, on-board selection.
Driver	Pal Kaszonyi	Logistics arrangements.
JDS ITR Electrofishing Team	Petra Stahlschmidt- Allner	Investigation on fish indicator species. Analysis of indicator species parazitisation.
	Bernhard Allner	Co-ordination between electrofishing and lab-based fish investigation, blood sampling.
JDS ITR Extended Electrofishing Team	Egbert Korte	Electrofishing taxonomic analyses.
	Zoltan Sallai	Electrofishing taxonomic analyses.
	Nadia Nikutowsky	Electrofishing.
	Annette Schaat	Sex determination, tissue sampling, tissue sampling management (storage registration).
	Silke Dehe	Sex determination, tissue sampling, tissue sampling management (storage registration).
	Andre Beltz	Electrofishing, taxonomic analyses tissue sampling.

# 3.2.2 National Teams

There are five adjacent Regional Environmental Inspectorates along the Hungarian Tisza section. Their names from the downstream section to the Upper Tisza are as follows:

Lower Tisza Valley Environmental Inspectorates, Szeged; Middle Tisza Valley Environmental Inspectorates, Szolnok; Trans-Tisza Environmental Inspectorates, Debrecen; North Hungarian Environmental Inspectorates, Miskolc; Upper Tisza Valley Environmental Inspectorates, Nyíregyháza. Hydrobiologists and chemists joined the different Tisza sections in order to carry out their routine sampling program in the given sampling locations. In the mean time they had the opportunity to follow and practice the sampling procedures that were used by the Core Team on the board, as well.

# 3.2.3 Sample types and treatment in the field

**Water** was sampled at each sampling site from the middle of the stream to carry out on-board analysis for the following components:

pH, dissolved oxygen, water temperature, electric conductivity, alkalinity, ammonium, nitrite, nitrate, orthophosphate, chlorophyll-a. **ITR 1 – ITR 22**: samples were taken using the facilities provided by the German laboratory-ship ARGUS. **ITR 23 – ITR 27**: samples were taken using cars; therefore the general parameters (temperature, pH, dissolved oxygen and conductivity) couldn't be recorded on the upper stretch. Due to the fact that on-board analyses were carried out immediately after sampling and preparation of water samples, no preservation method was used.

**Phyto-** and **zooplankton** samples from 27 locations of the Tisza River and its tributaries were collected. Lugol solution was used for the preservation of the plankton samples. Rotatoria and Crustacea (Cladocera, Copepoda) species were determined from filtered samples. Species composition and individual abundance values were determined for each sampling section. Based on the results assessment is given on the water quality of the river and its tributaries. Recent results are compared to former studies.

**Sediment, suspended solids**, **mussel** and **macroinvertebrate** samples were taken during the ITR from 27 sampling stations, 20 from the Tisza River and 7 from the main tributaries, respectively. Sampling at each station includes these four sample types, and three of them (i.e. sediment, suspended solids and mussel samples) were analysed according to the attached determinant list (*Table 2*). Surface layer of sediment was collected in the Tisza River and its tributaries at 27 sampling locations. Particularly those habitats were included that are characterised by significant sedimentation in order to pick up the finer sediment fraction than 63  $\mu$ m. Average sample was prepared from the two sub-samples taken at left and right at each given station cross sections. In case of the Tisza River (and its tributaries) the total mixing process happens in relatively short distance.

On-board grain size fractionation with wet sieving were done from the average sediment samples in order to receive fraction less than 63 microns, for later analyses of heavy metals, PAHs, PCBs and organo-chlorinated compounds similarly to the *JDS program.* Sample analysis was carried out in the VITUKI Laboratory.

Mussel species were collected from the sampling sites if there were living specimens. Direct diving, grabbing and dredging were used when picking up the mussel specimens from the inhabited zone of the Tisza and its tributaries. The same micropollutants compounds were determined in the mussels as it is described in case of the sediment and suspended solid samples (*Table 3.2*). Mussel samples were preserved by deep freezing for later analyses of heavy metals, PAHs, PCBs and organo-chlorinated compounds and they were analysed in the VITUKI Laboratory, as well.

The macroinvertebrate community structure was determined (relative abundance of taxa) and based on these taxonomic results, the Saprobity index was determined using the saprobic list of the Austrian Fauna Aquatica Austriaca (*Table 3.3*). Living macroinvertebrate samples were selected during on-board activity. The rest of the unfinished samples were preserved in the ethanol solution (70 %).

Sampling, sample processing and the results referring to the fish investigations are given in the Annex I.

Determinand	Unit	Method used	Limit of quantification [LOQ]	Accreditation [YES/NO]
Heavy metals				
Cadmium (Cd)	mg/kg	L-AAS	0.3	Yes
Chromium (Cr) – total	mg/kg	L-AAS	0.8	Yes
Copper (Cu)	mg/kg	L-AAS	0.5	Yes
Lead (Pb)	mg/kg	L-AAS	1.5	Yes
Mercury (Hg)	mg/kg	CW-AAS	0.002	Yes
Nickel (Ni)	mg/kg	L-AAS	0.7	Yes
Zinc (Zn)	mg/kg	L-AAS	0.2	Yes
Aluminum (Al)	mg/kg	L-AAS		Yes
Arsenic (As)	mg/kg	L-AAS		Yes
Organic compounds				
PAH – 16 EPA	mg/kg	SPE/GC/MS	0.005	Yes
PCB – 7 (each)	mg/kg	SPE/GC/MS	0.005	Yes
Lindane (gamma-	mg/kg	SPE/GC/MS	0.001	Yes
hexachlorocylohexane)				
Hexachlorobenzene	mg/kg	SPE/GC/MS	0.001	Yes
Hexachlorobutadiene	mg/kg	SPE/GC/MS	0.005	No
Pentachlorobenzene	mg/kg	SPE/GC/MS	0.005	Yes
pp'DDT	mg/kg	SPE/GC/MS	0.001	Yes

# Table 3.2. List of determinands in sediment, suspended solids and mussel samples including relevant additional information

Table 3.3. List of determinands in macroinvertebrate samples

Determinand	Method used	Instrumentation used
Macrozoobenthos (No. of Taxa)	Evaluation of semi- quantitative data	Stemi 2000 stereomicroscope
Macrozoobenthos (Sapr. Index)	Quantity measured on interval scale	Zelinka-Marvan method, saprobe values based on Fauna Aquatica Austriaca

# 3.2.4 On board analytical methods

# Preparation:

Water samples were filtered using membrane filtration (0.45 µm pore size); filters were stored at 4<sup>o</sup>C for later determination of suspended solids concentration; filtered water was used for on-board and laboratory analyses of nutrients

### Analyses:

General parameters (temperature, pH, dissolved oxygen - concentration and saturation - and conductivity) were recorded using the facilities provided by the German laboratory-ship Argus (on-line WTW equipment);

Alkalinity – analysis was carried out using unfiltered water samples, immediately after sampling, according to ISO 9963-1: Determination of total and composite alkalinity:

*Nutrients:* analyses were carried out using filtered water samples, immediately after filtration, according to the below mentioned methods:

N – NH<sub>4</sub>: ISO 7150/1: Determination of ammonium – manual spectrometric method;

N - NO2: Standard Methods for the Examination of Water and Wastewater sixteenth edition. Method no. 419:

N – NO3: ISO 7890-3 Part 3: Spectrometric method using sulfosalycilic acid;

P - PO4: Standard Methods for the Examination of Water and Wastewater sixteenth edition, Method no. 424 F, ascorbic acid method;

**Reagents and chemicals:** all the used on board reagents and chemicals were used according to the quality required by the above mentioned standards and were provided by VITUKI Institute.

### Instrumentation:

on-line WTW equipment for general parameters;

UV-VIS Spectrophotometer VARIAN 50 Carv for on-board analyses of nutrients

### **Quality control:**

Checking the amount of substance concentration of the hydrochloric acid used for determination of alkalinity - every two days;

Calibration curve for each nutrient parameter;

Control sample analyzed at the beginning of each working day for each parameter.

# 3.2.5 Biological methods

Samples for species diversity of phytoplankton were taken by plankton net (mesh size 10  $\mu$ m), while for the other analyses collected water samples were used. Identification of the cyanophytes/cyanobacteria and algae were done by light microscopy, abundancy (expressed as number of cells per 1 ml) was done by counting living organisms using Cyrus box. Content of chlorophyll-a was measured by two methods (ISO 10260 and by fluorimetry).

Zooplankton samples were taken from the surface. 50 liters of water was filtered through 70 µm mesh size plankton net at each sampling location. Upon the filtering of the sample animals attached to the inner side of the net were washed to the collection vessel by immersing the net several times to water. After this procedure the collected material was quantitatively poured to the sample container glass. Applying Lugol solution achieving "cognac" color of filtrate was used to preserve samples.

Species lists contain mainly the planktonic elements of Rotatoria and Crustacea (Cladocera, Copepoda). Nevertheless, there were tychoplanktonic species in the samples and species lists by chance. These were collected from the aqueous phase incidentally.

Dissection of Cladocera and Copepoda species was conducted under stereoscopic microscope in 9%-os glycerin solution with dissection needles. Dissected body parts were placed to appropriate position using 200-400 times magnification for the identification of the species. The sample was investigated by counting the animals in whole sample for the quantitative investigations. Investigations were conducted in 5 ml chambers using Utermöhl-type inverted microscope. Animal abundances are given in units of ind./100 liters. Taxon list containing the results of the qualitative investigations indicate frequently occurring and dominant species with *italics – bold* characters.

Species identification was based on the following taxonomic books: BANCSI 1986, 1988, NOGRADY 1993, SEGERS 1995, SMET and POURRIOT 1997, KOSTE 1978, KUTIKOVA 1970 (Rotatoria), FLÖSSNER 1972, GULYÁS 1974, GULYÁS and FORRÓ 1999, MANUJLOVA 1964 (Cladocera), DÉVAI 1977, DUSSART 1967, 1969, EINSLE 1993, GULYÁS and FORRÓ 2001 (Copepoda).

List of sampling sites is given in *Table 3.1.* Quantitative and qualitative results of zooplankton investigations are given in *Table 4.2.2.1* where **bold** character indicate the results of the tributaries. Last row of the tables contains the taxon number found in a given sampling location. Individual abundance values are given graphically also in *Figure 4.2.2.1*. Taxonomical determination of Rotatoria and Crustacea species was made by KATALIN ZSUGA(Middle Tisza Region Environmental Inspectorate, Szolnok).

Macroinvertebrates (including mussel species) were collected on the right and left banks, respectively. An average sample was created from these two sub-samples. Both the hydraulic Polyp grab of the ARGUS and the FBI hand net having a mesh size of 950  $\mu$ m were used for the sampling. In each cross section the combined data received by these two methods are taken into consideration. Qualitative taxon list illustrates presence/absence data. Relative abundance of taxa were expressed in a five step interval scale as follows:

- 1: present (1-2 individuum/sample);
- 2: rare;
- 3: frequent;
- 4: common;
- 5: very common, masses.

## 4 Results and discussion 4.1 Chemical status characterization

# 4.1.1 On board analysis of water

Results measured on-board concerning the water samples are summarized in *Table 4.1.1.1* (Yellow color indicates the tributary samples). The water temperature was in the range of 15-16,7 °C, the pH values indicate slightly alkaline medium. The dissolved oxygen is not far from 100 % saturation along the whole Tisza, icluding the tributaries, except the Bega (2,9 mg/l DO– 32 %).

The electric conductivity of the Tisza is around 300  $\mu$ S/cm, conductivity values of the tributaries are a little higher (maximum value in the Sajó: 550  $\mu$ S/cm). Alkalinity is smaller in the Tisza than in its tributaries (maximum value in the Zagyva: 7,3 mval/l).

The amounts of the main inorganic nutrient compounds are indicated in *Figures* 4.1.1.1-4.



Figure 4.1.1.1 Amount of NO<sub>3</sub>-N in the Tisza and its tributaries, ITR, 2001

The amount of nitrate nitrogen is slightly decreasing in upstream direction after the Kisköre Reservoir. Maximum values are shown in the Zagyva and the Sajó reaching 2,5 mg/l concentrations. The same tendency is shown on Table 4.1.1.2 where the nitrite nitrogen is illustrated. Tributaries such as the Bega and the Zagyva contained much more of this compound than the others and the Tisza itself (max. 0,066 mg/l).





There are no large differences in the concentration of ammonium nitrogen in the Tisza and its tributaries, except the Bega again (*Figure 4.1.1.3*) where the maximum value was measured (0,73 mg/l).







Figure 4.1.1.4 Amount of orto-PO<sub>4</sub>-P in the Tisza and its tributaries, ITR, 2001

The ITR sampling sites and four tributaries had the same amount of orthophosphate phosphorous. Three tributaries exceeded the average value (*Figure 4.1.1.4*): the Bega, the Sajó and the Zagyva. The last one was characterized by the large overall maximum value (0,526 mg/l).

It can be concluded that the amounts of the most important inorganic nutrient substances were not high in the Tisza River. There were only few tributaries where extreme values were detected like the Bega (ammonium and nitrite nitrogen) and the Zagyva (orthophosphate phosphorous) most probably due to permanent organic load. In case of orthophosphate phosphorous concentration the Sajó has a little bit increased value also, due to the communal wastewaters of Miskolc city but this is not as much as in case of the Zagyva at all.

In order to evaluate the nutrient load of the Tisza and its tributaries it seems to be worthwhile to take the concentration of chlorophyll-a in consideration, as well. The degree of trophity as the realized primary production in water due to planktonic eutrophycation can be explained this way.

In case of the Tisza River it is clear that the concentration of chlorophyll-a is not high, especially in the Upper Tisza stretch. The maximum value was measured at the downstream end of the Kisköre Reservoir. However, the Zagyva River with the detected 73  $\mu$ g/l chlorophyll-a concentration seems to be an outstanding water course in that respect, too. It looks like the key element in case of that explanation should be the phosphorous. The origin of o-phosphates is most probably the large number of villages and cities along the Zagyva that is characterized by small flow rate especially during late summer, early autumn.



Figure 4.1.1.5 The chlorophyll-a concentration along the Tisza and its tributaries

Station Country/			Km-	- Date of	Water	рΗ	Dissolved C	Dxygen	Cond.	Alk.	NH4-N	NO2-N	NO3-N	oPO4-P
No.	Countries	Town/Location or Tributary Name	Index	sampling	temp. (0C)		Mg/I	%	µS/cm	mval/l	mg/l	mg/l	mg/l	mg/l
ITR 1	YU	Titel, Danubian confluence	2	29.Sept	16,5	7,52	7,6	81	310	2,0	0,07	0,018	0,92	0,065
ITR 2	YU	Bega Navigable Canal	3	29.Sept	17,8	7,19	2,9	32	400	2,6	0,73	0,066	0,88	0,130
ITR 3	YU	Novi Becej	66	30.Sept	16,4	7,59	6,7	71	300	3,0	0,05	0,015	0,98	0,065
ITR 4	YU	Novi Knezevac	147	30.Sept	16,2	7,45	7,6	81	300	2,0	0,05	0,006	1,14	0,065
ITR 5	YU, H	Szeged, old city bridge	172	01.Oct	16.0	7,61	7,6	83	320	2,1	0,04	0,012	1,09	0,065
ITR 6	Η	Maros, Makó	178	01.okt	16,3	7,63	7,9	80	430	1,9	0,04	0,012	1,48	0,065
ITR 7	Н	Mindszent	216	02.Oct	15,6	7,64	6,8	70	280	2,0	0,06	0,012	0,83	0,065
ITR 8	Η	Hármas-Körös, Kunszentmárton	243	02.Oct	15,7	7,45	7,1	78	280	2,2	0,03	0,015	0,63	0,032
ITR 9	Н	Tiszaug, híd	267	03.Oct	15,5	7,69	8,4	84	270	2,0	0,03	0,012	0,92	0,065
ITR 10	Н	Szolnok	332	03.Oct	15,8	7,68	8,3	86	300	2,0	0,03	0,009	0,95	0,032
ITR 11	Η	Zagyva, Szolnok	336	03.Oct	-	-	-	-	-	7,3	0,03	0,057	2,42	0,586
ITR 12	Н	Kisköre	406	04.Oct	15,5	7,71	7,6	78	320	2,2	0,05	0,018	0,89	0,065
ITR 13	Н	Aranyosi-sziget (Kisköre Reservoir)	419	04.Oct	15,6	7,65	8,2	88	320	2,1	0,02	0,012	0,95	0,065
ITR 14	Н	Tiszafüred-Poroszló	431	04.Oct	15,5	7,67	8,4	90	310	2,2	0,03	0,009	0,92	0,065
ITR 15	Н	Tiszacsege	454	05.Oct	15,5	7,68	7,9	80	330	2,1	0,04	0,015	1,04	0,065
ITR 16	Н	Polgár	487	05.Oct	15,4	7,68	9,2	92	310	1,9	0,04	0,009	0,92	0,065
ITR 17	Η	Sajó, Kesznyéten	492	05.Oct	16,7	7,96	9,1	95	550	3,4	0,04	0,021	2,36	0,163
ITR 18	Н	Rakamaz-Tokaj	544	06.Oct	16,0	7,74	8,5	87	330	2,1	0,05	0,009	0,80	0,032
ITR 19	Η	Bodrog, Bodrogkeresztúr	544	06.Oct	16,2	7,51	7,4	79	310	2,4	0,10	0,024	0,90	0,065
ITR 20	Н	Tiszabercel	569	07.Oct	16,0	7,70	9,2	93	330	2,2	0,03	0,009	0,80	0,065
ITR 21	Н	Dombrád	592	07.Oct	16,2	7,71	8,5	89	340	2,1	0,02	0,006	0,75	0,032
ITR 22	Η	Tuzsér	615	07.Oct	16,2	7,73	8,6	90	360	2,2	0,02	0,003	0,74	0,032
ITR 23	Н	Aranyosapáti	675	08.Oct	-	-	-	-	-	2,2	0,04	0,015	0,78	0,032
ITR 24	Н	Gergelyiugornya	685	08.Oct	-	-	-	-	-	2,1	0,02	0,003	0,55	<0,005
ITR 25	Η	Szamos, Tunyogmatolcs	686	08.Oct	-	-	-	-	-	2,7	0,04	0,012	0,54	0,032
ITR 26	Η	Tivadar	705	08.Oct	-	-	-	-	-	2,0	0,01	0,003	0,51	<0,005
ITR 27	Н	Tiszabecs	744	08.Oct	-	-	-	-	-	2,0	0,01	0,003	0,47	<0,005

# Table 4.1.1.1 Results of the on-board water analysis during the ITR mission

# 4.1.2 Inorganic micropollutants in water

In order to demonstrate the heavy metal content, the analysis of the *Figure 4.1.2.1* should be given to show the suspended solid concentration during the ITR sampling program. Large concentrations were detected especially on the Lower and Middle Tisza stretches (max value in Maros and Tisza at Szeged) because high flood was characteristic in the first half of the sampling program. There was one order of magnitude higher suspended sediment load (80 mg/l) measured in the Maros that indicated the different watershed of the later river. This high rate of suspended solids explains the usual higher concentrations of inorganic compounds in the Maros River.



# Figure 4.1.2.1 Concentration of suspended solids during the ITR sampling program, 2001

Mercury, cadmium, lead, chromium, copper, nickel, zinc, aluminum, iron, manganese and arsenic were analyzed in water, suspended solids, bottom sediment and mussel tissue. The proposal of the MLIM Expert Group for the water quality classification based on the results of the Transnational Monitoring Network in the Danube River Basin includes target values for the dissolved and total concentrations of the following metals:

Metals	Unit	Target value (dissolved forms)	Target value (total)
Zinc	μg/l	5	100
Copper	μg/l	2	20
Chromium	μg/l	2	50
Lead	μg/l	1	5
Cadmium	μg/l	0.1	1
Mercury	μg/l	0.1	0.1
Nickel	μg/l	1	50
Arsenic	μg/l	1	5

Total mercury concentration was between >0.03 – 0.13  $\mu$ g/l in the Tisza river. The highest value was measured downstream of the confluence of the Maros tributary, which discharged very high (0.25  $\mu$ g/l) mercury concentration owing to the extreme large suspended solids content during flood period. The dissolved mercury concentrations were below the 0.1  $\mu$ g/l limit value.

The cadmium, lead, chromium, copper, nickel, zinc, aluminum, iron, manganese and arsenic total concentrations showed a similar longitudinal profile along the Tisza river as it was experienced for the mercury: moderately low values in the upper and middle section of the river and significant increase downstream of the Maros confluence. Only a minor part of the total metal content was in dissolved form. The dominant proportion of the metals was attached to suspended particles. The *Figures 4.1.2.2-12* illustrate the heavy metal content of original and filtered water samples in pairwise comparison.



Figure 4.1.2.2 Amount of Hg in the original water sample



Figure 4.1.2.3 Amount of Cd in the original water sample



Figure 4.1.2.4 Amount of Pb in the original water sample



Figure 4.1.2.5 Amount of Cr in the original water samples

The maximum value of chromium is due to the high suspended solid load of the Maros River at Szeged section at the sampling site. Similar high values of other heavy metal compounds are indicated in the other diagrams referring to the same reason (*Figures 4.1.2.2-12*).



Figure 4.1.2.6 Amount of Cu in the original and filtered water samples



Figure 4.1.2.7 Amount of Ni in the original and filtered water samples



Figure 4.1.2.8 Amount of Zn in the original and filtered water samples



Figure 4.1.2.9 Amount of AI in the original and filtered water samples



Figure 4.1.2.10 Amount of Fe in the original water sample



Figure 4.1.2.11 Amount of Mn in the original water sample



Figure 4.1.2.12 Amount of As in the original and filtered water samples

# 4.1.3 Inorganic micropollutants in suspended solids, sediment and mussels

IWACO (2000) proposed limit values for heavy metals in sediment of the Danube river basin:

Metals	Unit	Limit value in sediment
Zinc	mg/kg	200
Copper	mg/kg	50
Chromium	mg/kg	100
Lead	mg/kg	100
Cadmium	mg/kg	1
Mercury	mg/kg	0.5
Nickel	mg/kg	50
Arsenic	mg/kg	40

The heavy metal concentrations varied in a wide range depending on the type of the samples and the type of heavy metal. *Figures 4.1.3.1-11* summarize the different heavy metal concentrations in suspended solids, sediment and different mussel species collected in the Tisza (yellow) and its tributaries (red), respectively. Figures are grouped in one page indicating the amounts of the same pollutant measured in the three different investigated compartments. In case of mussels the use of logarithmic scale on Y axis is more demonstrative and advisable at the same time, because the differences in the order of magnitudes are illustrated only by that way.

Altogether the three most frequently found mussel species were selected for the inorganic and organic micropollutant analysis, depending on which of them was present in the given sampling location. Data on *Unio tumidus*, *U. pictorum* and *U. crassus* are available as the results of the ITR program referring to the Tisza and its tributaries.

*Mercury* (Hg) limit concentration was exceeded in the suspended solids and bottom sediment of the tributary Sajó. The amount of 1,3 mg/kg measured in the suspended solids is almost the double than in the sediment sample (0,75 mg/kg). It is a very clear phenomenon, which indicates historic (former) mercury pollution. During flooding the mixing up causes resuspension. Heavy metals could be remobilized from the deposited sediment layer and transported further downstream along the river at any time.

There are no important differences among the three commonly occurring mussel species in terms of the investigated mercury concentrations (*Figure 4.1.3.1*). It is interesting that a slight decrease is detected in the upstream direction. The range of mercury concentration is generally below 0,1 mg/kg. The tributaries represent the same degree of pollution; the Zagyva contains the lowest mercury value (in *Unio tumidus*).

The *cadmium* (Cd) concentration was dominantly well above the *1 mg/kg limit value* both in the suspended solids and sediments of Tisza River (3-5 mg/kg), a bit higher values were found in the sediment (*Figure 4.1.3.2*). The sediment of Szamos has the maximum Cd value (7 mg/kg). Higher amounts (approaching and exceeding 10 mg/kg) were measured in the different mussels especially in case of mussels

collected in the Sajó, too. Again lowering values are shown to the upstream direction of the Tisza. The values measured in the tributaries are similar but the Zagyva represents the minimum value in U. *tumidus*, as well.

*Lead* (Pb) measured in the suspended solids and sediments also exceeded the limit collected in some sampling sites in the Tisza and in tributaries Szamos and Maros. The amount of lead in the soft tissues of mussels falls in a range of 10-25 mg/kg on the lower Tisza stretch. It decreases upstream direction both in case of the Tisza and its tributaries (*Figure 4.1.3.3*).

*Chromium* (Cr) concentrations are close to the limit, copper levels are mostly above the proposed limit value. A very similar picture can be seen concerning the chromium distribution along the river (*Figure 4.1.3.4*). Values vary about 10-25 mg/kg on the lower stretch. They are around few mg/kg in the middle and the upper section. Maximum value among the upper half of the investigated stretch was measured in the mussels collected in the Sajó (*Unio pictorum*, 15,7).

*Copper* (Cu) varied between 50-180 mg/kg both in suspended solids and sediment, the lower values were characterizing the middle section of the river. Its amount was below 100 mg/kg in the soft tissues of all mussel species everywhere (*Figure 4.1.3.5*).

*Nickel* (Ni) concentrations were around the 50 mg/kg limit: in the fine particle size suspended sediment they were just above that, in the bottom sediment below the limit value. The amount of nickel in mussels is not significant neither in the Tisza nor in its tributaries (*Figure 4.1.3.6*).

Typical *zinc* (Zn) concentrations in the Tisza are two times higher than the limit. Zinc occurs in the mussel tissues in considerable amount (*Figure 4.1.3.7*). It is clear from the diagram that larger concentrations were detected in the lower Tisza (Yugoslavia) and in the Kisköre Reservoir. Maximum values approach 2000 mg/kg dry weight, indicating that this compound causes serious pollution problem in the whole region.

*Figure 4.1.3.8* illustrates the amount of *aluminum* (AI) measured in the different type of samples. It varies between 25000-40000 mg/kg (suspended solids), 15000-30000 mg/kg (sediment), but it is between 100 and 1000 mg/kg in the soft mussel tissue samples.

*Arsenic* (As) concentration was above the limit only in the sediment of the tributary Szamos. It is characteristically present in the Tisza River basin. The diagram (*Figure 4.1.3.9*) shows that its amount in the mussel soft tissues is not significant (range: 1-0,1 mg/kg). The lowest value was detected in the mussels collected in the Zagyva.



Figure 4.1.3.1 Concentration of mercury (mg dry weight/kg) in different compartments



Figure 4.1.3.2 Concentration of cadmium (mg dry weight/kg) in different compartments



Figure 4.1.3.3 Concentration of lead (mg dry weight/kg) in different compartments



Figure 4.1.3.4 Concentration of Cr (mg dry weight/kg) in different compartments



Figure 4.1.3.5 Concentration of copper (mg dry weight/kg) in different compartments



Figure 4.1.3.6 Concentration of nickel (mg dry weight/kg) in different compartments



Figure 4.1.3.7 Concentration of zinc (mg dry weight/kg) in different compartments



Figure 4.1.3.8 Concentration of AI (mg dry weight/kg) in different compartments


Figure 4.1.3.9 Concentration of arsenic (mg dry weight/kg) in different compartments



Figure 4.1.3.10 Concentration of Mn (mg dry weight/kg) in suspended solids and sediment



### Figure 4.1.3.11 Concentration of iron (mg dry weight/kg) in suspended solids and sediment

Generally it can be concluded that the investigated triplet of compartments as suspended solids, sediment and mussel tissues contained different amounts of inorganic pollutants as follows: the suspended solid compartment contains always bigger amount of any heavy metal component than the sediment. Usually both compartments have bigger metal contamination than the mussels, except two metals. Only the Cd and the Zn occurred in higher amounts in the investigated mussel species than in the suspended solid and sediment phase. The amount of mercury was the same in the sediment phase and in the mussel tissue. The average concentration values are illustrated in *Table 4.1.3.1*.

### Table 4.1.3.1 Average values of different metal components in suspended solid, sediment and mussel compartments (mg/kg dry weight)

	Hg	Cd	Pb	Cr	Cu	Ni	Zn	AI	As
Susp. solids	0,2	3,3	88,7	93,9	125,7	55,4	511,5	32350,0	24,4
Sediment	0,1	2,6	64,0	56,2	90,7	45,6	430,3	20792,6	19,4
Mussels	0,1	5,8	6,1	5,2	36,1	1,8	769,3	583,4	0,5

#### 4.1.4 Comparison of heavy metal data to other results

Some heavy metal concentration values referring to the mussel tissues are shown for comparison in *Table 4.1.4.1* where the measurements of the Yugoslavian laboratories are given. They found similar figures in case of Hg, Cd, Ni and Zn. They measured lower concentrations in case of Pb, Cr and Cu. It can be concluded that this type of common sampling program is one of the best possibility to harmonize different methods and approaches.

Heavy metal compounds were monitored by OERTEL (1996, 1997, 2000a, 2000b) using Dreissena polymorpha as an on-site monitoring kit in the Hungarian Danube section.

According to the 7-week period exposure time higher concentrations of Cd, Pb and Ni were measured in Dreissena mussels in the Danube than in Unionidae in the Tisza and its tributaries. However, the amount of Zn and Cu in the Tisza mollusks proved to be much bigger than in the experiments of OERTEL.

Several possibilities are given for the comparison of heavy metal content of sediment and mussel tissues regarding those data that were collected during the Bioindicator study (VITUKI/ICPDR 2000). In case of mercury the order of magnitude is the same in the mussel species. There were a little higher values measured in the sediment especially from Pancevo section (maximum 0.55 mg/kg Hg in the Danubian sediment) comparing to the average Tisza pollution level (0.1 mg/kg). The measured maximum mercury concentration in the Sajó is highly similar to the maximum value detected in the Velika Morava in 2000.

The Cd-level was similar to the ITR measurements in the sediment (2-3 mg/kg) and in case of the mussels, as well (max.: around 10 mg/kg in the Tisza mussels, 8,4 mg/kg in *Unio pictorum* collected in Tekija). Generally the Cd-content of mussel tissues increased in the Danube downstream direction, especially after Belgrade section, in the Iron Gate Reservoir. These values did not change too much along the Tisza River.

The lead and chromium contents of the Danubian sediment and mussels were a little bit higher than in case of the Tisza. Copper concentrations were bigger in the sediment samples and the mussels collected in the Tisza, some Danubian values reached the relevant Tisza figures only in the downstream section. Same statement can be given for the distribution of nickel: both values are very similar to each other in the Danube and Tisza, respectively.

	1	2	3	4
Hg	<0.06	<0.06	<0.06	<0.06
Cd	8,7	0,5	4,0	6,0
Pb	8,7	5,5	2,5	5,5
Cr	7,5	<2.5	<2.5	2,5
Cu	27,5	6,0	35,7	55,0
Ni	<2.5	<2.5	6,7	<2.5
Zn	1050,0	325,0	222,0	525,0

# Table 4.1.4.1 Heavy metals measured in mussels (mg/kg dry weight) by the Yugoslavian partners on the lower ITR section (ITR 1-4)

#### 4.1.5 Organic micropollutants

#### Polar pesticides

Atrazine, simazine, propazine and endosulfan were analysed to estimate the effect of agricultural pesticide application. From the four pesticides atrazine, simazine and propazine were found above detection concentration (>1 ng/l), endosulfan concentrations were below detection level.

The target limit value for atrazine is 100 ng/l in the MLIM EG water quality classification proposal for the TNMN. Atrazine concentrations were between < 1-66 ng/l in the Tisa River and in the tributaries.

The highest atrazine concentration was measured in the Tisa at Szeged (rkm 174), downstream of the Maros confluence. The Maros had also elevated (48 ng/l) atrazine concentration, presumably owing to the intensive surface runoff from arable land in the Maros river basin.

For the other analyzed polar pesticides (simazine, propazine, endosulfan) target values are not available in the MLIM proposal for the TNMN.

For the four analyse pesticides the LAWA German water quality targets for aquatic communities and for drinking water supply are as follows:

Table 4.1.5.1	LAWA water quality target for aquqtic life and drinking water
	supply

Pesticide	Quality ta	rget LAWA (ng/l)				
	for aquatic life	for drinking water supply				
Endosulfan	5	100				
Propazine	-	100				
Simazine	100	100				

The simazine concentrations were less than 10 ng/l in the Tisa river and in the tributaries. In the downstream section of the Tisa higher concentrations were found than in the upper stretch.

The propazine concentrations were also in the range < 1-10 ng/l, like simazine.

During JDS atrazine and simazine were analyzed, propazine and endosulfan analyses were not part of JDS. The mean atrazine concentration was around 50 ng/l in the Danube. The 100 ng/l target value was exceeded in a few samples. The comparison shows that the atrazine concentration levels were slightly lower in the Tisa than in the Danube. In the Tisa River and in its tributaries the target limit value was not exceeded.

Simazine was not found in JDS samples (limit of determination was 7 ng/l in JDS). In the Tisa river and in the tributaries simazine concentration was also less than 7 ng/l in most of the samples.







Figure 4.1.5.1 Atrazine, simazine and propazine in the water of Tisa and its tributaries

Organochlorine compounds, polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs)

The above pollutants tend to be accumulated in solid phase therefore they were analyzed in suspended solids, sediment and mussels.

The applied limit values for their quantitative characterization were selected from Rhine and Elbe sediment quality criteria and from Canadian guidelines because there is no sediment classification system for the Danube river basin (*Table 4.1.5.2*).

Component	Unit	Limit value (target value or maximum allowable conc.)	Remark									
Lindane	μg/kg	10	Rhine target									
Hexachlorobenzene	μg/kg	40	Rhine target									
Hexachlorobutadiene	μg/kg	20	Dutch target									
Pentachlorobenzene	μg/kg	-	-									
pp'-DDT	μg/kg	40	Elbe ARGE (Elbe target)									
PCBs	μg/kg	20 (each)	LAWA Germany									
PAH	ua/ka	780	Canadian sediment quality									
(benzo(a)pyrene)	μy/ky	700	guideline									

 Table 4.1.5.2
 Limit values of organic pollutants in sediment

Except of hexachlorobutadiene all the other pollutants or groups of pollutants are present in the suspended solids, sediments and mussels.

*Figure 4.1.5.2* indicates the distribution of the studied pollutants in the different matrices (sediment, suspended solids, mussel) by the ratio of their mean concentrations in mussel/sediment and in mussel/suspended solid.



## Figure 4.1.5.2 Concentration ratio of pollutants in mussel/sediment and in mussel/suspended solids

The figure shows that mussels concentrated some of the pollutants. The accumulation factor was around 10 for the components PCB 153, PCB 138, pentachlorobenzene and lindane.

*Figures 4.1.5.3 – 4.1.5.26* illustrate the organic micropollutant content of suspended solids, sediment and mussel.

#### Organochlorine compounds

<u>pp'-DDT</u> was found in the sediment of the Tisza River and in the tributaries in low concentration (mean in Tisza 0,0058 mg/kg, mean in the tributaries 0,0027 mg/kg), compared to the 0,040 mg/kg limit value.

In the suspended solids the pp'-DDT concentrations were much higher than in the sediment. Both in the tributaries and in the Tisza River the mean concentrations exceeded the limit value (0,040 mg/kg). Although DDT application was banned for several decades in the Tisza River Basin, a high concentration of the suspended solids can be explained by the soil erosion of cultivated lands.

In the mussels the pp'-DDT mean concentration was 8,5  $\mu$ g/kg. It is somewhat higher than the mean pp'-DDT concentration in Tisza sediment (5,8  $\mu$ g/kg), but much lower than the mean pp'-DDT concentration in the Tisza suspended solids (50  $\mu$ g/kg).

<u>Lindane</u> concentrations in the Tisa River and in the tributaries were very low both in the sediment and in the suspended solids. The mean concentrations were around 10 % of the Rhine target value (10  $\mu$ g/kg). Lindane was accumulated in mussels, the mean lindane concentration of mussels was 10.2  $\mu$ g/kg.

The mean <u>pentachlorobenzene</u> concentrations were similar to the lindane concentrations both in the sediment, suspended solids and mussels.

<u>Hexachlorobenzene (HCB)</u> concentrations were typically less than the lindane or pentachlorobenzene concentrations in sediment and suspended solids. Compared to the target value (40  $\mu$ g/kg) this pollution level is negligible. In mussel the HCB content was below the detection level.

<u>Polychlorinated biphenyls (PCBs)</u> concentrations were typically below the 20  $\mu$ g/kg target value, except PCB 28 in suspended solids and sediment in the tributaries Sajó and Bodrog, and the Tisa River at Tiszabercel (rkm 569).

The representative compound benzo(a)pyrene of <u>polyaromatic hydrocarbons (PAHs</u>) varied in the concentration range  $<2 - 160 \mu g/kg$  in suspended solids and sediment. Even the highest concentrations (measured in suspended solids at Szolnok (rkm 332) and at Tiszazug (rkm 267) were less than half of the Canadian guideline value. Benzo(a)pyrene concentrations in mussels were lower than in suspended solids or sediment.

The mean concentration levels of the above noted pollutants were similar in the Danube and in the Tisa during the JDS and ITR surveys.





Figure 4.1.5.3. Lindane and PCB 153 in suspended solids







Figure 4.1.5.4 Pentachlorobenzene, HCB and DDT in suspended solids













Figure 4.1.5.6 Lindane and DDT in sediment







Figure 4.1.5.7 PCB-138, PCB-28 and HCB in sediment





#### Figure 4.1.5.8 Pentachlorobenzene and benzo(a)pyrene in sediment

Similarly to the inorganic micropollutant analyses the three most frequently found mussel species were selected for the organic micropollutant analysis, depending on which of them was present in the given sampling location. Data on *Unio tumidus, U. pictorum* and *U. crassus* are available as the results of the ITR program referring to the Tisza and its tributaries. Next figures summarize the different organic compounds (pesticides, PCBs, PAHs) in different mussel species collected in the Tisza (yellow) and its tributaries (red), respectively. In this case too, the use of logarithmic scale for Y axis is more demonstrative because only the important differences (order of magnitudes) are illustrated by this way.



Figure 4.1.5.9 Concentration of naphthalene (ng/g) in mussels, ITR 2001



Figure 4.1.5.10 Concentration of fluoranthene (ng/g) in mussels, ITR 2001



Figure 4.1.5.11 Concentration of benzo(b)fluoranthene (ng/g) in mussels, ITR 2001



Figure 4.1.5.12 Concentration of benzo(a)pyrene (ng/g) in mussels, ITR 2001



Figure 4.1.5.13 Concentration of benzo(k)fluoranthene (ng/g) in mussels, ITR 2001



Figure 4.1.5.14 Concentration of indeno(1,2,3-c)pyrene (ng/g) in mussels, ITR 2001



Figure 4.1.5.15 Concentration of benzo(g,h)perylene (ng/g) in mussels, ITR 2001



Figure 4.1.5.16 Concentration of pp'-DDT (ng/g) in mussels, ITR 2001



Figure 4.1.5.17 Concentration of lindane(gamma-hexachlorocyclohexane (ng/g) in mussels



Figure 4.1.5.18 Concentration of pentachlorobenzene (ng/g) in mussels, ITR 2001



Figure 4.1.5.19 Concentration of sum of PCB's (ng/g) in mussels, ITR 2001



Figure 4.1.5.20 Concentration of PCB180 (ng/g) in mussels, ITR 2001



Figure 4.1.5.21 Concentration of PCB28 (ng/g) in mussels, ITR 2001



Figure 4.1.5.22 Concentration of PCB52 (ng/g) in mussels, ITR 2001



Figure 4.1.5.23 Concentration of PCB101 (ng/g) in mussels, ITR 2001



Figure 4.1.5.24 Concentration of PCB118 (ng/g) in mussels, ITR 2001



Figure 4.1.5.25 Concentration of PCB153 (ng/g) in mussels, ITR 2001





Identification of organic components

GC-MS scans identified the following components in water and sediment at sampling stations ITR-1(Titel, Danubian confluence) and ITR-5 (Szeged, rkm 172):

• Organic compounds in water (together with suspended solids):

aliphatic hydrocarbons having different number of carbon atoms, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzanthracene, benzofluoranthenes, benzopyrenes, DDT, molecular sulphur

• Organic compounds in bottom sediment:

aliphatic hydrocarbons having different number of carbon, phenanthrene, fluoranthene, pyrene, diisobutyl phthalate, pentadecanal, hexacosanal, 4,4-dimethyl cholestanone, sulphur.

The identified compounds are typical natural and anthropogenic components of water and sediment.

#### 4.2 Ecological status characterization

#### 4.2.1 Phytoplankton

It has to be mentioned that phytoplankton species diversity, abundancy and chlorophyll-a were relatively low from station Titel (the confluence of the Tisza River to the Danube) upstream the whole Tisza River. The reason of that was the flooding situation and high water level.

Characterising **phytoplankton species diversity** of the Tisza and its tributaries, all together 177 taxa were identified. The number of taxa ranged between 20 – 80 in the Tisza River, while it varied from 15 to 77 in the tributaries. The highest number of phytoplankton species was found at Mindszent (80) among all stations of the Tisza River. Number of taxa identified on-board during ITR within individual groups of phytoplankton in Tisza and its tributaries are presented in *Figure 4.2.1.1*. It can be said that from Tiszabecs to Mindszent number of taxa slightly increased. Downstream stations of Tisza were influenced by high water level since the beginning of the ITR sampling program.

Generally, the diatoms (Bacillariophyceae), green algae (Chlorococcales) and Euglenophyceae were the most dominating taxa. The minor groups were Cyanophyceae/Cyanobacteria, Dinophyceae, Conjugatophyceae, Xanthophyceae and Cryptophyceae. In some stations (in Tiszacsege, Dombrád, Szamos tributary, Tiszabecs) there were more taxa of Chrysophyceae than of Euglenophyceae. The most often species were Chrysococcus rufescens, Mallomonas acaroides, Dinobryon divergens, Synura uvella (Chrysophyceae), Aulacoseira granulata, Cyclotella meneghiniana, Melosira varians, Stephanodiscus hantzschii, Asterionela formosa, Diatoma vulgaris, Fragilaria ulna var. ulna, Navicula cryptocephala, N. Lanceolata, Nitzschia acicularis, N.palea (Bacillariophyceae). Green coccal algae were represented mostly by Coelastrum astroideum, C. microporum, Pediastrum duplex, Scenedesmus acuminatus and S. opoliensis. Among the Crypophyceae the genera of Cryptomonas occured freequently. Organic pollution was indicated by the presence of euglenoids (mostly Euglena caudata, E. pisciformis, Strombomonas verrucosa, Trachelomonas hispida, T. planctonica, T. volvocina, T. volvocinopsis). The list of taxa of the phytoplankton is given in Annex of the report.



#### Figure 4.2.1.1. Number of species for individual phytoplankton group of Tisza River and its tributaries.

**Phytoplankton abundancy** was expressed by the number of cells per ml. It can be stated than during the ITR the abundancy of phytoplankton increased from Tiszabecs to Kisköre. Other downstream sites were influenced by high water level. Zagyva and Sajó were eutrophic tributaries having the highest values of abundancy (31404 cells per ml, resp. 40484 cells per ml) within all the measured stations. This was indicated by the high chlorophyll-a values, too. The abundancy of the phytoplankton is shown in *Figure 4.2.1.2*. The distribution of the individual group of cyanobacteria and algae is presented in *Figure 4.2.1.3*. It is evident that diatoms were the most abundant algae in the whole investigated stretch of the Tisza and as well as in its tributaries. Green coccal and flagelate algae created second abundant group from Titel to the confluence of Bodrog. In the upper part of the Tisza (from Sajó confluence) numbers of cells of Chrysophyceae increased.



Figure 4.2.1.2. Phytoplankton abundance (cells per ml) in the investigated stations of Tisza and its tributaries



# Figure 4.2.1.3. Distribution of abundance of the individual groups of cyanobacteria and algae within the ITR stations

Phytoplankton biomass was expressed by the content of chlorophyll-a. Based on obtained results the concentration of chlorophyll-a was generally low in Tisza River. Highest values were measured in the tributaries, mainly in Zagyva and Sajó which is in harmony with phytoplankton abundancy. Biomass was measured by two methods. The differences between the two methods for the whole investigated stretch can be seen in *Figure 4.2.1.4*. Generally the results obtained by ISO method were higher.

The content of chlorophyll-a was highly influenced by high content of suspended solids due to the high water level.



# Figure 4.2.1.4. Content of chlorophyll-a (μg/l) in the Tisza River and its tributaries measured during ITR

#### 4.2.2 Rotatoria and crustacea zooplankton

The international expedition on the Tisza River conducted in September 2001 collected zooplankton samples from 27 locations of the Tisza River and its tributaries. Rotatoria and Crustacea (Cladocera, Copepoda) species were determined from filtered samples. Species composition and individual abundance values were determined for each sampling section. Based on the results assessment is given on the water quality of the river and its tributaries. Recent results are compared to former studies.

#### Literature survey

Survey of the Hungarian zooplankton studies concerning stagnant and flowing water bodies revealed that the Tisza river and its tributaries, dead-arms, ox-bows, and claypits, as well as the Kisköre reservoir on the river has been investigated historically in details.

The first papers on the Tisza were published by DADAY (1888, 1891) and ÉBER (1955). Investigations on clay-pits were published by VARGA (1928, 1930). Most detailed data on the river and its dead-arms was published by MEGYERI (1955, 1957, 1959, 1961, 1970, 1971, 1972). Description of the medium section was done by GÁL (1963) and BANCSI (1976b, 1977) while benthic Rotatoria and Entomostraca fauna was discussed by BANCSI, HAMAR (1981) and ZSUGA (1981, 1997b).

Results of the detailed and periodical Rotatoria and Crustacea investigation conducted in 1992 on the Tisza and its tributaries were published by GULYÁS ET AL. (1995). The authors investigated regularly 12 sections on the Tisza.

As the results of this complex series of investigations it was concluded that in general the planktonic elements dominated the river, most of which is cosmopolitan, adopted species. Large number of described taxa (altogether 181 Rotatoria, 28 Cladocera and 8 Copepoda) is also noteworthy.

There are many publications concerning the smaller streams, dead-arms, inundated areas of the Tisza catchment area (VARGA 1931, 1953, 1966, BANCSI 1974, 1976a, 1980, ZSUGA 1981, 1999, ZSUGA and NAGY 1989, 1991). Water quality questions of the catchment area and ecological considerations of the Kiskörei reservoirs were discussed by ÁDÁMOSI et al. (1974, 1978), BANCSI and VÉGVÁRI (1990), as well as ZSUGA, BANCSI and VÉGVÁRI (1990).

Zooplankton communities of the Kiskörei reservoir were investigated in details during the past decade. Investigations covered the aspects of quantitative and qualitative survey of zooplankton, monitoring of seasonal dynamics, and characterization of mosaic patterns and biodiversity (ZSUGA 1994, 1995, 1996, 1998, 1998a, ZSUGA, BANCSI 1995, ZSUGA, EGYED, KRUPINSZKI 1995). Rotatoria and Crustacea fauna of the densely vegetated water bodies, stands of different basins, as well as biotecton components on the macrovegetation was also investigated in details (ZSUGA 1996, 1996a, 1996b, 1997, ZSUGA, BANCSI, VÉGVÁRI 1998). Investigations into the fish food resources of the seaweed and wetland vegetation were also conducted lately (SZÍTÓ, ZSUGA, BANCSI, KOVÁCS, VÉGVÁRI 1997).

During the investigations related to the cyanide spill from Romania in January 2000 and later on studies revealed the occurrence of 306 species of Rotatoria, Cladocera and Copepoda. Highest taxon number was measured at Tiszafüred (60), while lowest at Tiszasziget (35). There were many rare species identified, particularly from the Tisza River occurrence, which was not recorded previously. The primary habitat of these species is, however, not the plankton, but the water body within the aquatic macrovegetation or the surface layer of the sediment. Their presence in the water body is the result of the runoff and drift.

In the period of cyanide pollution most of the plankton was destroyed, approximately 70-80 % of Rotatoria and Cladocera species was damaged. Following the washdown of the toxic plume in a few days some species were represented in small individual abundances, particularly the members of high tolerance species. Further increase of species number and individual abundances was small due to a flood event in the middle of February.

Following the cyanide spill the results of investigations throughout 2000 indicated that the individual abundances of the stands varied within large ranges. On the basis of this the Tisza could be classified into three sections. The upper section to the confluence point of the Szamos (1-2183 ind./10 I), following the section to the Kiskörei Reservoir (3-7154 ind./10 I), and finally, further downstream to the southern state boundary (8-9592 ind./10 I).

Results of the investigations upstream of the Kiskörei reservoir on the Tisza indicated that according to the flood periods the representatives of the investigated zooplankton groups (Rotatoria, Cladocera, Copepoda) were present in small species numbers and individual abundances in most of the samples. Following the cessation of the flood the individual abundances of the zooplankton species grew spectacularly. The runoff water from the floodplains drifted many, rarely occurring organisms into the plankton of the Tisza river. These taxa were, however, represented occasionally and only in isolated river sections.

On the basis of the results it is concluded that the re-growth of the plankton community of the river started quite soon, following only a few days amongst the changing conditions.

The heavy metal pollution that occurred in March did not influence the microfauna of the middle Tisza significantly. Individual abundances were rather effected by a new flood. There are many species in the plankton that were drifted from the sediment to the aquatic phase (*Dicranophorus caudatus, Encentrum eurycephalum, Lophocharis salpina, Paradicranophorus hudsoni, Paracyclops fimbriatus*). The presence of these species supports the hypothesis that organisms survived in the sediment and the regeneration of the aquatic ecosystem of the river did started soon not only in the aqueous phase but also in the sediment and sediment surface.

Surveying the results of the investigations lead to the conclusion that the zooplankton of the Tisza River changed significantly after the cyanide spill compared to the results of the previous 10-15 years. This was particularly expressed in the period following the floods when both the species composition and individual abundances were different in 2000 than in previous time period under the low water conditions. This could be ascribed to various mechanisms of the intoxication processes, followed by a large flood as well as to the high mortality of filter feeder fish population (silver carp). Results of the investigation series conducted from the end of February until the end of October indicate that the "ordinary" zooplankton changed in species composition and individual abundances. Occurrence of rare species and occasional population increase during some seasons, far above the regular values was observed with regards to zooplankton stands. There might be multiple reasons behind this phenomena but the direct and indirect effect of the intoxication could not be excluded as potential factors.

Results of the investigations prove that ecological conditions changed significantly that was followed by a quick repopulation of the plankton of the Tisza. In this pivotal role is attributed to the non-polluted tributaries, floodplains and Kiskörei reservoir as well. Following the low diversity initial period richer plankton was formed both in terms of qualitative and quantitative character. The composition of the newly formed community is influenced by climatic factors as well as the water level fluctuations of the river and prevailing general hydrological conditions.

Comparison of the results of quantitative investigation also shows that similarly high individual abundances were measured as early as 1992 along the longitudinal section of the Tisza. From Záhony to Tiszasziget similar values of individual abundances were measured between June-August in this year.

Large zooplankton stands were observed in the Csenger section of the Szamos, at Buj on the Lónyai channel, at Tokaj on the Bodrog, at Kesznyéten on the Sajó and at Makó on the Maros as far as the tributaries are concerned. In 2000, however, only in the maros we measured similar high individual numbers.

In the middle and lower Tisza region many dead-arm was investigated on nature protection areas. Results indicated that many rare zooplankton species registered here, rarely occurring in Hungary (Gói-tói-, Csatlói-, Feketevárosi-, Mártélyi-Holt Tisza, Sulymos). These areas are of high priority nature protection areas, their further protection is emphasized herein (VITUKI 2001).

#### Evaluation of results

Investigations on the longitude of the Tisza river and its tributaries revealed the presence of 41 Rotatoria, 6 Cladocera and 8 Copepoda, altogether 55 taxa. The reason for the small taxon number is the high water level on the rivers causing very high suspended solid load at some locations (Tisza: Titel, Novi Becej, Novi Knezevac, Szeged, Szamos Tunyogmatolcs, Tiszafüred, Tiszacsege, Gergelyiugornya, Tivadar) This problem made the identification of species and counting of individual numbers critically difficult (*Table 4.2.2.1*).

Primarily the number of Cladocera species was low and only a few species was determined that are characteristic to Tisza plankton: *Bosmina* (*B.*) *longirostris, Disparalona rostrata, Moina micrura*. Amongst the rare species only the occurrence of *Alona guttata* was recorded. The species number of Copepods was slightly higher with characteristically occurring species *Acanthocyclops robustus* and *Eucycloops serrulatus*. Occurrence of *Eurytemora velox* is noted specially at the Kunszentmárton sampling site of the Hármas Körös. This particular species was only found in the Danube and in waters west to the Danube in Hungary so far.

Highest species number was found amongst the Rotatoria taxa, such as *Brachionus angularis, B. budapestinensis, B. calyciflorus, Keratella cochlearis cochlearis, K. c. tecta, Lecane bulla, Polyarthra dolichoptera, Synchaeta oblonga* and *S. pectinata* species belonging to the dominant elements of the Tisza cacthment system. These species are often occurring in slow flowing and stagnant eutrophicated Hungarian waters, forming large stands under favorable conditions. The members of *Brachionus* and *Synchaeta* genera are tolerating organic pollution as well. In late summer the occurrence of predatory *Aspanchna* species was often recorded in high numbers. Number of rare species (*Ascomorpha ecaudis, Cephalodella biungulata, Colurella colurus, C. uncinata, Encentrum saundersiae, Lepadella rhomboides*) was lower than during former investigations due to flood conditions this time. Of these the following are mentioned: These are tychoplanktonic species with primary habitat in the surface of sediment or the water body among the aquatic vegetation. These species were drifted to the real plankton.

The Rotatoria and Crustacea plankton of the Tisza along the longitudinal section was characterized by the dominance of the following species in late September – early October 2001: Brachionus angularis, B. budapestinensis, B. calyciflorus, Keratella cochlearis cochlearis, K. c. tecta, Polyarthra dolichoptera, Synchaeta oblonga, S. pectinata, Bosmina (B.) logirostris, Acanthocyclops robustus.

**Individual abundaces** were much less than during lower water level periods. Individual numbers per 100 liters varied between 8-12144 in the Tisza, and 12-10704 in the tributaries. Along the longitudinal section of the Tisza this number was actually between 8-560 and the only large maximum was measured in one occasion at Tiszacsege sampling site. The same is concluded for the tributaries where the largest single value was observed in the Zagyva at Szolnok sampling site. In both occasion some eutrophic Rotatoria species and nauplius and copepode larvae of Copepods grew out of proportion (*Figure 4.2.2.1, Table 4.2.2.1*). These values are smaller by one or in some cases by two orders of magnitude compared to the results of previous studies conducted during summer, late summer periods. This conclusion is particularly true for the Kiskörei reservoir and for sections upstream and downstream of the reservoir. Low individual numbers are particularly striking in the lower Tisza section at Tiszaug, Mindszent, Szeged regions where at least two magnitudes of orders higher abundances were measured formerly throughout the year. Low individual numbers are the results of the flood.



Figure 4.2.2.1 Abundance of zooplankton during the ITR sampling program on the Tisza

Individual abundance values on the upper river section are used to be higher by one magnitude of order but their longitudinal distribution is similar to the one experienced during previous years: in the section between Tiszabecs and Dombrád (rkm 744-592) abundances are usually low, downstream of this section (rkm 569-431) this value increases considerably. In 1992 for example maximum values were found on this section on the Hungarian stretch of the river. The reason for this is the impoundment effect of the Tiszalök hydroelectric power dam and Kiskörei reservoir. Downstream of this the individual abundance monotonously increases towards to the Hungarian-Yugoslavian border. This was not evidenced by the longitudinal investigations. Effects of the tributaries on this section usually elevate individual numbers such as the Hármas Körös and Maros rivers. The flood masked this effect during the period of investigation.

Amongst the other tributaries the Rotatoria and Crustacea plankton of the Sajó near to confluence point showed the usual mesotrophic state (11 species, 623 ind./100 liter) with dominant species characteristic to eutrophic waters. The medium polluted water of Zagyva primarily indicates organic material pollution at Szolnok. High individual numbers of *Brachionus-, Keratella-, Polyarthra,- Synchaeta*-Thermocyclops species is recorded with zooplankton numbers 10704 ind./100 liters. The reason for this is the slow flowing character of the river and highly available concentrations of plant nutrients.

The other reason of low species and individual numbers is the low availability of food for the filter feeder Rotatoria and Crustacea species as it is evidenced by the chlorophyll a concentration values. Chlorophyll-a concentration value ranged 0,41-4,12  $\mu$ g/l on the Tisza upstream of the Kiskörei reservoir and 1,84-5,04  $\mu$ g/l downstream of the reservoir. No longitudinal increase was practically observed along the river in chlorophyll-a contents.

The two highest values were measured at the confluence point of the Sajó (15,98  $\mu$ g/l) and at Szolnok in the Zagyva (732,93  $\mu$ g/l), where zooplankton stand numbers were also high.

**Summarizing** the results of the ITR sampling program conducted in Septemberearly October it is concluded in general that only 55 Rotatoria and Crustacea species lived in the Tisza and at the confluence points of its tributaries – in lower than usual values. Dominant species were however identical to previously observed ones: *Brachionus angularis, B. budapestinensis, B. calyciflorus, Keratella cochlearis cochlearis, K. c. tecta, Polyarthra dolichoptera, Synchaeta oblonga, S. pectinata, Bosmina (B.) logirostris, Acanthocyclops robustus.* Occurrence of rare species became even more seldom compared to the results of previous investigations.

Individual abundance values of the animals are usually lower by one-two orders of magnitude than in previous years during the same season (late summer – early fall). No increase in individual numbers was observed due to impoundment and on downstream sections. One possible reason for this is the narrow food resources for the filter feeder zooplankton species as it is evidenced by the measured low chlorophyll a concentration values.

ТАХА										San	npling	site	s									
		5	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	23	24	25	26	27
ROTATORIA																						
Anuraeopsis fissa (Gosse, 1851)													16									
Ascomorpha ecaudis Zacharias, 1893									8													
Asplanchna priodonta Gosse, 1850				6			48			8	5056											6
Bdelloideasp.							96	32	8		64		16					16			36	6
Brachionus angularis angularis (Gosse 1851)							144	16	8		64				4							
<b>B. budapestinensis budapestinensis</b> Daday, 1885							48															
B. calyciflorus calyciflorus Pallas, 1766						12	384	16														
<i>B. c.anureiformis</i> Brehm, 1909	8				2	16	576															
<i>B. c. spinosus</i> Wierzejski,1891							48				128											
B. diversicornis diversicornis (Daday, 1883)										8	64											
<i>B. quadridentatus ancylognathus</i> Schmarda, 1859							48															
<i>B. q. cluniorbicularis</i> Skorikov, 1894								16														
<i>B. q. rhenanus</i> Lauterborn, 1893							384						16									
<i>B. urceolaris</i> (O.F.Müller, 1773)							48				64											
Cephalodella biungulata Wulfert, 1937							48															6
Cephalodella sp.						4																
Colurella colurus (Ehrb., 1830)													16									
C. uncinata (Müller, 1773)																						6
Encentrum saundersiae (Hudson, 1885)											64											
Euchlanis dilatata Ehrb., 1832						4	48															
Filinia longiseta (Ehrb., 1834)							48															
Keratella cochlearis cochlearis (Gosse, 1851)		8		6		8	1824	32	40		1600	8	32		28	8						
<b>K. c. tecta</b> (Gosse, 1851)			4	6		40	2544	64	16	8	2688	32	48	32	32							
K. quadrata (O.F.Müller, 1786)											64											
Lecane (Monostyla) bulla (Gosse, 1886)					2	8						24	176		4							
L. (Monostyla)closterocerca (Schmarda, 1859)				6		4		16		24	128	32	16	96		4		8		6		
<i>L. luna</i> (O.F.Müller, 1776)								16							4							
L. (Monostyla) lunaris (Ehrb., 1832)																8						
Lepadella rhomboides (Gosse, 1886)													32									
Notholca squamula (O.F.Müller, 1786)							48															
Polyarthra dolichoptera Idelson, 1925							432		40		832			16	32							

### Table 4.2.2.1. Quantitative data of zooplankton taxa during the ITR sampling program (ind/100l)

ТАХА										San	npling	site	s									
		5	7	8	9	10	11	12	13	14	15	16	17	18	20	21	22	23	24	25	26	27
P. major Burckhardt, 1900											192											
P. vulgaris Carlin, 1943											64						8					
Pompholyx sulcata Hudson, 1885											64											
Synchaeta oblonga Ehrb., 1831					4		1296	96	216	40	448			288	172	20	8					
S. pectinata Ehrb., 1832							1200		16					16	8							
Trichocerca birostris (Carlin, 1943)						4							176									
<i>T. pusilla</i> (Lauterborn, 1898)						4	48	16														
Trichocerca sp.					2		48															
Trichotria curta (Skorikov, 1914)		8																	12			
T. pocillum (Müller, 1776)															4							
Sum:	8	16	4	24	10	104	9408	320	352	88	11584	96	544	448	288	40	16	24	12	6	36	24
CLADOCERA																						
Alona guttata Sars, 1862																						
Alona sp.												8		16								
Bosmina (Bosmina) Iongirostris (O.F.Müller, 1785)					2	4					32	8										
Ceriodaphnia sp.															4							
Disparalona rostrata (Koch, 1841)					6								31									
Moina micrura Kurz, 1874											16											
Sum:	0	0	0	0	8	4	0	0	0	0	48	16	31	16	4	0	0	0	0	0	0	0
COPEPODA																						
Acanthocyclops robustus (Sars, 1873)					10			32														
Cyclops strenuus strenuus Fischer, 1851					6																	
Cyclops sp.		16				4																
Eucyclops serrulatus (Fischer, 1851)					4																	
E. speratus (Lilljeborg, 1901)						4																
Eurytemora velox (Lilljeborg, 1853)					4																	
Macrocyclops albidus (Jurine, 1820)																						
Thermocyclops crassus (Fischer, 1853)							192															
nauplius Cyclopoida			4	18	14	24	1008	104	24	32	448	16	32	64	12	4		8	12	6	12	
copepodit Cyclopoida	16			18	50	28	96	40	16		64	8	16	32	8							
Sum	16	16	4	36	88	60	1296	176	40	32	512	24	48	96	20	4	0	8	12	6	12	0
Sum zooplankton	16	16	8	60	106	168	10704	496	392	120	12144	136	623	560	312	44	16	32	24	12	48	24
Number of taxa:	1	3	1	4	10	13	22	11	8	5	18	6	11	6	10	4	2	2	1	1	1	4
#### 4.2.3 Macroinvertebrates

The first benthological surveys on the Tisza started in the beginning of the XX. century dealing with Mollusks (ROTARIDESZ, 1926, 1927a, 1927b, CZÓGLER, 1927, 1935). Several studies can be listed dealing with the aquatic snails and mussels of the Tisza River after the middle of this century (HORVÁTH 1940, 1943, 1955, 1958, 1962, 1965, 1972, BÁBA 1967, 1974, 1978; FERENCZ 1968; ÁDÁMOSI et al. 1978; B. TÓTH and BÁBA 1980, 1981; SZÍTÓ and BOTOS 1989, 1993, 1994). The analysis of the collected material of KOLOSVÁRY and MAJOROS forms the base of the early Oligochaeta results (ZICSI 1965, FERENCZ 1968, 1969, 1974a,b, 1981); Further literature is available after ÁDÁMOSI et al. (1978); FERENCZ and SZÍTÓ (1980); SZÍTÓ and BOTOS (1989).

The Malacostraca taxa of the Tisza are reported by LANTOS (1986). Publication on *Palingenia longicauda*, the most famous species of the Tisza River are found after FERENCZ (1968, 1974a, 1974b); CSOKNYA and HALASY (1974). TÓTH (1966a, 1972, 1974), SZÍTÓ and BOTOS (1989) publish several data on Odonata larvae and imagoes. FERENCZ (1974b) reports data about Trichopteran taxa, especially on the Lower Hungarian Tisza section.

Very valuable information on Diptera species living in the river is summarized by TÓTH (1966b, 1967, 1969). FERENCZ (1968) started the description of the Chironomidae species, later on SZÍTÓ (1972, 1978, 1979, 1987, 1995, 1996); ÁDÁMOSI et al, (1978), FERENCZ (1974b); FERENCZ and SZÍTÓ (1980); and SZÍTÓ and BOTOS (1989) published several results on this taxonomic group. PUJIN (1988) and PUJIN et al. (1984, 1986, 1987) describe the faunal changes of the Yugoslavian Tisza section caused by modified hydrological conditions.

Several long sectional sampling programs of the Tisza River are reported recently dealing with the faunal composition: AMBRUS et al. (1995a, 1995b, 1996, 1998); JUHÁSZ et al.(1998, 1999, 2000); KOVÁCS et al. (1998, 1999a, 1999b, 1999c, 2000); NESEMANN and CSÁNYI, (1993); CSÁNYI et al. (1996); VARGA and CSÁNYI (1997, 1998-1999).

Before evaluating the results of the macroinvertebrate samples two main points should be emphasized. Firstly, the taxonomic results reflect to the relatively late sampling period. Due to the autumn season, several taxon groups were absent from the Tisza and its tributaries during the ITR mission. Those hemihydrobiont insect taxa that hatch usually earlier in spring and summer were almost not detectable already, like numerous known and formerly described species of the mayflies (Ephemeroptera) and stoneflies (Plecoptera) groups. The exact description of those species that were still detectable in the Tisza River in earlier seasons one year after the cyanide spill is given in the Report on the Cyanide pollution and its biological effects (VITUKI 2000, 2001).

Secondly, there was a coincidental high flood during the sampling mission that caused several difficulties in sampling. The water level of the Tisza has increased considerably. Flooding always causes very serious difficulties in sampling of such a large river as the Tisza. Therefore a new sampling method was introduced in the Tisza investigations. The motorboat of ARGUS was used for dredging the different

habitats of the rivers situated at deep water. Dredging is a difficult issue, especially when it should be done in deep water conditions. It requires much effort and care, at the same time. However, the results suggest that finally this method was applied very successfully.

Altogether 107 macroinvertebrate taxa were found during the ITR sampling program (*Table 4.2.3.1*). The distribution of the main characteristic taxa is illustrated in diagrams where the relative abundance estimated in a five degree ordinal scale is given.

The total number of Mollusca taxa is relatively high (26) compared to the other taxon groups, especially if the taxon number of the still not completely determined group (Chironomidae, 23) is not taken in consideration. The lowering water temperature can explain the absence of some mussel species from the very upstream section of the Tisza. VITUKI data showed their continuous presence from this stretch long ago. In case of decreasing water temperature, as the summer period is over, the animals are migrating to the deeper parts of the river bottom. Therefore it is difficult to find them due to their extremely low population density.

Several Gastropoda taxa are abundant along the whole Tisza River (*Lithoglyphus naticoides, Bithynia tentaculata, Viviparus acerosus, Valvata naticina*). The population of *Theodoxus fluviatilis* is permanently expanding toward upstream of the river during the last decade (VITUKI 1992). Ten years ago this organism was known only from three localities along the Tisza (ITR 5,6 and 10). At the present ITR study it was described from Dombrád (ITR 21) also. This species is present in abundant populations from the Danubian confluence and enters several tributaries like Bega, Zagyva and Bodrog.

The snails and mussels distribute the Tisza from the tributary, ITR 1 (Titel) until the ITR 22 (Tuzsér) section. On the upstream section there is only one exception (*Figure 4.2.3.1*) because of the presence of one snail species (*Lithoglyphus naticoides*) that was found at ITR 26 (Tivadar).

The most dominant species of the snails in the Tisza River is *Lithoglyphus naticoides*. The abundance values indicate that this species forms the most frequent snail population along the whole investigated stretch (the maximum abundance value of 5 appears at 4 sites). *Theodoxus fluviatilis* is a frequent member of the snail group with a medium frequency: larger populations are characteristic only on the downstream section. The species is detected in Dombrád (ITR 21) that represents the uppermost locality. *Viviparus acerosus* is the next present species in the decreasing order of magnitude: downstream sections have a little bit bigger populations than the middle and upper ones. Finally the fourth snail species in the decreasing order of abundance is *Borysthenia naticina* that generally occurs together with *L. naticoides* in several rivers (i.e. Danube).

*Figure 4.2.3.2* illustrates the distribution of the most dominant mussel species belonging to the genus *Unio*. Starting from Titel (ITR 1) to upstream direction, generally *Unio pictorum* was found in the largest number. The presence of *U. tumidus* is very even also because it can be found in considerable number usually during the whole investigated Tisza section. The distribution of the *U. crassus* is just

opposite to the *U. pictorum*. Largest populations were detected rather on the upper stretches. The maximum population size were characteristic for the upper four sections (ITR 18, 20, 21, 22). At the uppermost location (in Tuzsér) only *U. crassus* was found with a relatively big abundance value.



# Figure 4.2.3.1 Distribution and relative abundance of aquatic snails in the Tisza River

The other *Unionidae* species are relatively rare in the Tisza River (*Figure 4.2.3.3*). The Chinese pond mussel (*Sinanodonta woodiana*) is present on the lower and middle Tisza, it populates the Bega in large number. Its uppermost occurrence was detected in the Kisköre Reservoir. *Pseudanodonta complanata* shows similar pattern to the *Unio crassus*. Earlier data indicate their co-occurrence on the Upper Tisza section (at Szatmárcseke, 712 km, VITUKI, 2000). *Anodonta anatina* and *A. cygnea* are rare species in the Tisza, each of them has only two localities in the distribution map, mainly in the lower and middle stretches.



Figure 4.2.3.2 Distribution and relative abundance of frequent mussels in the Tisza River



Figure 4.2.3.3 Distribution and relative abundance of rare mussels in the Tisza River

Some Amphipoda taxa migrating from the Danube have large populations in the Tisza until the upper section (*Figure 4.2.3.4*). *Dikerogammarus villosus* has larger population density up to Kisköre (ITR 12) than *D. haemobaphes* but on the stretch Polgár-Tiszabercel (ITR 16-20) this later one becomes predominant among the Crustacea species. *Obesogammarus obesus* was detected sporadically in the lower and middle section. *Gammarus balcanicus* occurs only on the uppermost sections of the Tisza (from Dombrád until to Tiszabecs, ITR 21-27).



Figure 4.2.3.4 Distribution and relative abundance of Amphipoda in the Tisza River

Other Crustaceans represent the most crucial and characteristic species in the Tisza and its tributaries, because the damage of two of them by the cyanide spill was well documented (*Figure 4.2.3.5*). On one hand, the instantaneous (acute, on-site) detection of mortality of this species was examined and described at Mindszent (ITR 7) in February 2000. Relevant data are given in the first Cyanide Report (VITUKI, 2000). On the other hand, the total absence of *Corophium curvispinum* was proved on middle section of the Tisza shortly after the cyanide spill. Prior to the pollution the uppermost locality for this species was described at ITR 16. Comparing to these earlier data it is well indicating that the recolonization of the investigated Tisza section by the species *Corophium curvispinum* was successful during the last one and a half year period. High relative abundance values were found on the Tisza strech, especially up to ITR 10. Further expansion of this species is seen to the upstream direction because the presence data show that ITR 21 contains this species, too.

The presence of *Jaera istri* illustrates the same successful recolonization. This species prior to the cyanide spill was not detected above Csongrád section at all (VITUKI 2000, 2001). This time it was found above this point at four further locations:

Tiszaug, Szolnok, Kisköre and Tiszafüred, too. *Astacus leptodactylus* is a common member of the Crustacea fauna in the Tisza River, especially on the middle strech, similarly to the previous data.



Figure 4.2.3.5 Distribution and relative abundance of other Crustaceans in the Tisza River

The data on the Crustacea fauna illustrates well that all of those species that were damaged during the cyanide spill could recover completely. The distributed section of the Tisza by *Corophium curvispinum* and *Jaera istri* increased comparing to earlier data. Additionally, the abundance of these species has increased, too.

Altogether three characteristic rheophilous insect groups are taken to illustrate the distribution of them along the Tisza River. The season of autumn is not the most appropriate period to detect mayfly larvae in rivers but finally 12 taxa were identified from the investigated section (*Table 4.2.3.1*). This value compared to the overall number of 43 described from the Hungarian Tisza (VITUKI 2000, 2001) is not very big but indicate that some of them are characteristically occurs only on the Upper Tisza (Figure 4.2.3.6). *Heptagenia flava* was found on the middle stretch between Tiszaug and Dombrád (ITR 9-21) at 5 locations. The Tisza Flower (*Palingenia longicauda*) larvae were detected only at three Tisza sites (ITR 15, 21,22) having the hard clay substrate on the river bank. *H. longicauda* and *Caenis pseudorivulorum* both were found on the Upper Tisza strech only at three locations, as well.

The capture data of *Ametropus fragilis* is very illustrative for the indication of the late season problem. Normally (springtime) this species colonizes evenly the whole Hungarian Tisza, similarly to many other species. During the ITR program it was found mainly on the lower stretch of the river that is very unusual. Those species that were collected are relatively rare ones in other rivers of Hungary (*Baetis fuscatus, B.* 

tricolor, Procloeon bifidum, Ecdyonurus sp., Heptagenia longicauda, Paraleptophlebia submarginata, Cercobrachys minutus, Caenis pseudorivulorum).

The distribution of the Tisza flower (*Palingenia longicauda*) shows that during the fast moving sampling program only few colonized sites were detected in the ITR program (Maros, the Kiskörei Reservoir, the upper stretch at Dombrád and Tuzsér). The registered data are convincing about the fact that the *Palingenia*-colonies are in very well condition now.

It can be concluded that the taxon number of mayflies is much lower in autumn than at earlier periods of the year, and the occurrence data of most of them are restricted rather to the upper river stretches.

All of the four species of the Gomphidae family are living in the Tisza River but their distribution shows very interesting picture, as well (*Figure 4.2.3.7*). The most wide spread is *Stylurus (Gomphus) flavipes* that is found almost along the whole Tisza section (between ITR 3 and 24). *G. vulgatissimus* is also common species, co-occurring with the former one at several localities. However, two species are characteristically restricted to the uppermost stretches (*G. cecilia* and *G. forcipatus*).



Figure 4.2.3.6 Distribution and relative abundance of mayflies in the Tisza River



Figure 4.2.3.7 Distribution and relative abundance of Odonata larvae in the Tisza River

The distribution of the four detected caddis larvae of the genus Hydropsychidae is similar to the dragonflies (*Figure 4.2.3.8*). *H. bulgaromanorum* is the dominant one with a wide spread presence along the Tisza River (between ITR 3 and 22) in large relative abundance. *H. contubernalis* is not so frequent species. There are two species again that present only on certain stretches. *H. exocellata* was found on the Lower and Upper, *H. pellucidula* on the Upper Tisza River, respectively.



Figure 4.2.3.8 Distribution and relative abundance of caddis larvae in the Tisza River

Summarizing the faunal results it can be concluded that the autumn season was not very perfect for the collection of leeches. Only 7 leech species were detected this time, almost only from the Bega and the Sajó Rivers. The decreasing water temperature can explain the low number of leeches, because similarly to the mussels they start hiding themselves before winter.

The species number of damselflies and dragonflies are 9. *Stylurus (Gomphus) flavipes* is widespread species on the sections having extended fine sediment depositions along the riverbed. *Gomphus vulgatissimus* is also common, the two other Gomphidae taxa (*Ophiogomphus cecilia, Onycogomphus forcipatus*) occurred only on the uppermost section of the Tisza (ITR 26-27).

Stonefly species were found only in very restricted number (2!) also, due to the very inappropriate season for their perfect sampling. They could be detected between December and April in large numbers but strictly on the Upper Tisza stretches. The detected *Isogenus nubecula* is always relatively common here.

Saucer bug (*Aphelocheirus aestivalis*) represents the aquatic bugs by this time only in the Sajó. Its presence earlier was proved several times on the upper Tisza (VITUKI, 2000, 2001). There are interesting Coleoptera species occurring in the Tisza, on the lower, middle and upper stretch as well.

The caddis fauna (Trichoptera) is present almost along the whole river system. *Hydropsyche bulgaromanorum* is the most common but only the *H. contubernalis* and H. pellucidula reach the uppermost Tisza sections. *Neureclipsis bimaculata* and

*Ecnomus tenellus* were present in several locations. Some caddis species occurred only in the upper restricted stretch of the river (*Ceraclea nigronervosa, Oecetis notata*).

The analysis of the midges is not finished yet, so their number is going to be still increasing most probably. At present, 23 Chironomidae species are determined, most of them on the upper section.

Table 4.2.3.2 Number of	macroinvertebrate taxa in the main taxonomic groups
in samples	collected during the ITR mission, 2001.

ITR Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Gastropoda	4	8	5	6	3	0	2	4	2	3	8	3	5	4	3	5	2	3	9	2	2	3	0	0	0	1	0
Bivalvia	7	2	6	6	5	0	5	7	3	3	3	7	8	4	5	5	6	3	4	4	4	3	0	0	0	0	0
Malacostraca	4	1	6	4	4	2	4	3	4	6	0	4	3	4	3	4	4	2	3	3	4	2	0	1	0	1	1
Ephemeroptera	0	0	1	0	1	2	1	0	1	1	0	0	0	2	2	0	1	0	0	0	6	1	1	5	3	4	1
Odonata	0	1	1	1	2	2	2	1	0	1	0	0	2	0	1	1	1	1	3	3	3	2	1	5	5	1	2
Plecoptera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	1	2
Trichoptera	1	0	4	2	4	4	3	3	3	2	1	3	3	2	2	5	4	2	4	3	4	4	1	0	5	1	2
Number of taxa/sampling site	16	12	23	19	19	10	17	18	13	16	12	17	21	16	16	20	18	11	23	15	24	15	3	13	13	9	8

*Table 4.2.3.2 and Figures 4.2.3.9 and 4.2.3.10* show the taxonomic composition of the main macroinvertebrate groups along the Tisza and its tributaries. Only the main groups are considered, Chironomidae are not included because the analysis is still going. It is clear that Molluscs (snails and mussels) are almost absent from the upper section of the Tisza. Only *Lithoglyphus naticoides* occurred in Tivadar (ITR 26). There are no detected snails and mussels in the Maros and Szamos rivers at all. At the same time the group of insects dominates on this upper Tisza stretches. This conclusion is valid even in this case of late autumn season although many characteristic insect taxa are not present already, due to their earlier hatching time.



Figure 4.2.3.9 Number of macroinvertebrate taxa belonging to the main taxonomic groups at different sampling sites on the Tisza River

There were no Plecoptera taxa present at all in any of the tributaries. The highest number of the main taxonomic groups (6) was found in the River Sajó, that was formerly the most polluted Hungarian river. Due to the industrial collapse in both Slovakian and Hungarian regions, the water quality and the river fauna improved a lot during the recent decade.



Figure 4.2.3.10 Number of macroinvertebrate taxa belonging to the main taxonomic groups at different sampling sites on the tributaries

The same faunal composition of the Tisza can be seen from the more general picture when only three main taxonomic groups, Mollusca, Malacostraca and Insecta are presented (*Figure 4.2.3.11*). It is clear from the appearing mollusk taxa where the middle stretch of the Tisza River starts. The characteristic Upper Hungarian Tisza represents only a very restricted spatial extension that is found only upstream the Szamos confluence.



Figure 4.2.3.11 Number of macroinvertebrate taxa belonging to the main taxonomic groups at different sampling sites on the Tisza River

# Table 4.2.3.1 Relative abundance of macroinvertebrate taxa collected during<br/>the ITR mission, 2001.

ITR NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Puhatestűek (Mollusca)																											
Theodoxus fluviatilis (LINNAEUS, 1758)	2	2	3	3	3		2	2	3	2	2	2	1	2	2	2			3		2						
Viviparus acerosus (BOURGUIGNAT, 1862)		2	2	2				1		1				2					1	1		1					
Lithoglyphus naticoides (C. PFEIFFER, 1828)	5	1	4	4	4		4	3	3	3	2	4	5	5	4	3	3	5	5	4	2	2				3	
Bithynia tentaculata (LINNAEUS, 1758)		2	1	3	2						2			1					2								
Valvata naticina (MENKE, 1845)	1		2	2				1			2		1		1	2	1	2									
Valvata piscinalis (O. F. MÜLLER, 1774)		2									2																1
Acroloxus lacustris (LINNAEUS, 1758)												1	1			1			1			1					
Lymnaea auricularia (LINNAEUS, 1758)																			1								
Lymnaea peregra var.ovata (DRAPARNAUD)		2											2					1	1								
Physa acuta DRAPARNAUD, 1805		1		1							2					1			1								
Anisus spirorbis (LINNAEUS, 1758)											2																
Planorbarius corneus (LINNAEUS, 1758)	1	1																	1								
Planorbis planorbis (LINNAEUS, 1758)											1																
Kagylók (Mollusca: Bivalvia)																											
Unio crassus RETZIUS 1788			1		1		2	1	3	2	1	2	2	2	1	1	1	3	1	3	3	3					
Unio pictorum (LINNAEUS, 1758)	2		4	4	3		2	2				2	3	3	3	2	3	3	2	2	1						
Unio tumidus RETZIUS 1788	3		3	2	2		2	3	2	3		3	2	3	3	3	2	2	3	1	2						
Anodonta anatina (LINNAEUS, 1758)												1	1				1										
Anodonta cygnea (LINNAEUS, 1758)	1														1												
Sinanodonta woodiana (LEA, 1834)	1	3	1		1		2	1				1	1														
Pseudanodonta complanata															4	4					4						
(ROSSMASSLER, 1835)				1							1	1	1		1	1				1	1						
Dreissena polymorpha (PALLAS, 1771)	2	2	4	2	3		3	1	2	2		3	2	1		2											
Pisidium amnicum (O. F. MÜLLER, 1774)	1		1	1															2			2					
Pisidium henslowanum (SHEPPARD, 1823)													1									1					
<i>Pisidium moitesserianum</i> (PALADILHE, 1866)																	1										1
Sphaerium corneum (LINNAEUS, 1758)								1																			
Sphaerium rivicola (LAMARCK, 1815)	1			1				1			1						1										
Kevéssertéjűek (Oligochaeta)																											
Oligochaeta sp.	2	4	3	3	2	2	2	3						3	3	2	2	3	2	3	3	2				1	
Piócák (Hirudinea)																											
Hemiclepsis marginata (MÜLLER, 1774)																			1								
Glossiphonia complanata (LINNAEUS, 1758)		1		1																							
Alboglossiphonia heteroclita (LINNAEUS, 1761)		2																	1								1
Alboglossiphonia hyalina (MÜLLER, 1774)																			2								
Piscicola geometra (LINNAEUS, 1761)												1															
Erpobdella octoculata (LINNAEUS, 1758)		1																	2								
Helobdella stagnalis (LINNAEUS, 1761)		2																	2								
Rákok (Crustacea)																											
Limnomysis benedeni CZERNIAVSKY 1882			1							1																	
Corophium curvispinum (SARS, 1895)	2		4	4	4	2	4	2	4	5		2	2	2	3	4	4	3	3		1						
Dikerogammarus haemobaphes (EICHWALD, 1841)	3		1	3	2		2	2	2	2		2	2		2	3	3	3	2	3	2	2					

ITR NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Dikerogammarus villosus (SOVINSKY,		2	4		2	4	4	2	n	2			2	2		2	2			2	2						
1894)		2	4	4	3	1	4	2	2	3		4	2	3		2	2			2	2						1
Gammarus balcanicus SCHAFERNA, 1922																					1	1		1		2	2
Obesogammarus obesus (SARS, 1894)				2			1			1																	1
Asellus aquaticus (LINNAEUS, 1758)			1																2								
Jaera istri VIEUILLE, 1979	1		3		3				1	2		1		2													1
Astacus leptodactylus ESCHSCHOLZ, 1823	1													1	1	2	2			1							
Kérészek (Ephemeroptera)																											
Ametropus fragilis ALBARDA, 1878			1		2	1	1							1													
Baetis fuscatus (LINNAEUS, 1761)																										1	
Baetis tricolor TSHERNOVA, 1928																					2				1	2	
Procloeon bifidum (BENGTSSON, 1912)																								1			
Ecdyonurus sp.																								1			2
Heptagenia flava ROSTOCK, 1877									1	1				1	2						1				1		1
Heptagenia longicauda (STEPHENS, 1836)																					2			1	1	1	1
Paraloptophlebia submarginata (STEPHENS, 1835)																								1			
Ephemera vulgata LINNAEUS, 1758																	1										
Palingenia longicauda OLIVIER, 1791						1									1						2	2					
Cercobrachys minutus (TSHERNOVA, 1952)																					1		1				
Caenis pseudorivulorum KEFFERMÜLLER, 1960																					1			1		3	
Szitakötők (Odonata)																											
Calopteryx splendens (HARRIS, 1782)						1	1														1			1	1	3	1
Platycnemis pennipes (PALLAS, 1771)																			2	1				1	2		
Stylurus flavipes (CHARPENTIER, 1825)			3	2	2	2	2			1			2		2	1		1		2	3	2	1	1	2		
Gomphus vulgatissimus (LINNAEUS, 1758)					2			1					1				2			1	2	1		1	3		
Ophiogomphus cecilia (FOURCROY, 1785)																								1	2		
Onychogomphus forcipatus (LINNAEUS, 1758)																											2
Ishnura elegans pontica SCHMIDT, 1938																			2								
Coenagrion puella (LINNAEUS, 1758)																			2								
Orthetrum albistillum (LINNAEUS, 1758)		1																									
Álkérészek (Plecoptera)																											
Isogenus nubecula NEWMAN, 1833																					2			1		2	2
Leuctra sp.																								1			2
Poloskák (Heteroptera)																											
Aphelocheirus aestivalis (FABRICIUS, 1803)																	1										
Bogarak (Coleoptera)																											
Macronychus quadrituberculatus (P.W. J.MÜLLER. 1806)					1	1										1	1										
Potamophilus maculatus						1																		1			
Tegzesek (Trichoptera)																											
Hvdropsyche contubernalis MCLACHLAN.						•	•					-					•								•	•	
1865 Hydropsyche contubernalis masovica						3	3									1	2								3	2	2
MALICKY, 1977																									1		
Hydropsyche bulgaromanorum MALICKY, 1977			2	3	3	3	3	2	3	3		2	2	1	3	4	3		1	2	3	4					
Hydropsyche exocellata DUFOUR, 1841			1		1	1															2				1		
Hydropsyche pellucidula CURTIS, 1834								1								1	2										1
Hydropsyche sp.			3		3	2														1							1

ITR NO.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Neureclipsis bimaculata (LINNAEUS, 1758)	1		2	1	1		1	1	1	1		4	2	1	2	2	2	1	2	2	2	3	2		2		
Holocentropus dubius (RAMBUR, 1842)																1											
Holocentropus picicornis (STEPHENS, 1836)																			1								
Ecnomus tenellus (RAMBUR, 1842)									1		1	1	1					3	1								
Ceraclea nigronervosa (RETZIUS, 1784)																						1					
Oecetis notata (RAMBUR, 1842)																					1	1			2		
Kétszárnyúak <i>(Diptera)</i>																											
Ceratopogonidae sp.						1									1					1			1				
Chironomidae sp.		1		1	-	1					1				1			1	1	2	2	1	1				
Telopelopia fascigera (VERNEAUX, 1970)																							1			1	
Brillia longifurca									1															3		2	
Cricotopus sylvestris (FABRICIUS, 1794)									1																		
<i>Gymnometriocnemus brumalis</i> (EDWARDS, 1929)					1																						
Orthocladius obumbratus JOHANNSEN, 1905									1																		
Rheocricotopus effusus (WALKER, 1856)																										1	
Rheocricotopus fuscipes (KIEFFER, 1909)																										1	
Chironomus muratensis																					1						
Chironomus nudiventris																1	3	5		5	1					1	
Chironomus obtisidens GOETGHEBUER, 1921			2																1	2	1					2	
Chironomus plumosus (LINNAEUS, 1758)		1									2										1						
Chironomus riparius MEIGEN, 1804																							1			1	
Dicrotendipes nervosus (STAEGER, 1839)									1																		
Glyptotendipes glaucus (MEIGEN, 1818)																		1		5							
Glyptotendipes pallens MEIGEN, 1804											3																
Glyptotendipes sp.						1																					
Paratendipes intermedius																					1						
Paracladopelma laminata (KIEFFER, 1921)																								1			
Polypedilum pedestre MEIGEN, 1804																		1		2							
Stictochironomus pictulus (MEIGEN, 1830)																					1		2			2	
Stictochironomus sticticus (FABRICIUS, 1781)																					1		2	1		2	
Lipiniella moderata						2															2					1	
Kiefferulus tendipediformis GOETGHEBUER, 1921											2																
Psychodidae lárva																										1	

Data in *Table 4.2.3.1* show that the tributaries have several macroinvertebrate taxa, as well. The main importance is given to the faunal results of the Szamos River because the cyanide spill arrived through that river course to the region. Although the Mollusca and Crustacea fauna of the Szamos is very poor (it was very poor even before the cyanide spill!) other taxonomic groups like insects are represented in large species numbers. Mayflies, dragonflies and damselflies, caddis larvae have to be considered as normal faunal elements of the Szamos River. These results are in high concordance with the results of earlier hydrobiological monitoring data (VITUKI 2000, 2001).

# 4.2.4 Multivariate analysis of macroinvertebrates

Multivariate methods for hierarchical classification and ordination of sampling sites of the River Tisza, as objects based on the macroinvertebrate fauna provide appropriate division of different sections of the river. Three similarity indices (Jaccard, Sorensen and Chord distance) resulted in very similar dendrograms after the cluster analysis (*Figure 4.2.4.1*). The biplot of ordination gives similar division of the investigated Tisza section to the classification result (*Figure 4.2.4.2*). Table 4.2.4.1 shows the serial numbers of the Tisza sampling sites in order to the identification of the objects during different multivariate analyses.

Station No.	No.	Town/Location or Tributary Name	Km- index	Station No.	No.	Town/Location or Tributary Name	Km- index
ITR 1	1	Titel, Danubian confluence	2	ITR 15	11	Tiszacsege	453
ITR 3	2	Novi Becej	66	ITR 16	12	Polgár	498
ITR 4	3	Novi Knezevac	147	ITR 18	13	Rakamaz-Tokaj	544
ITR 5	4	Szeged, old city bridge	174	ITR 20	14	Tiszabercel	569
ITR 7	5	Mindszent	216	ITR 21	15	Dombrád	592
ITR 9	6	Tiszaug, híd	266	ITR 22	16	Tuzsér	616
ITR 10	7	Szolnok	334	ITR 23	17	Aranyosapáti	669
ITR 12	8	Kisköre	404	ITR 24	18	Gergelyiugornya	685
ITR 13	9	Aranyosi-sziget (Kisköre Reservoir)	419	ITR 26	19	Tivadar	705
<b>ITR 14</b>	10	Tiszafüred-Poroszló	431	<b>ITR 27</b>	20	Tiszabecs	744

Table 4.2.4.1 Serial number and ITR nur	mber of sampling sites on River Tisza
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Instead of ITR numbers, the serial numbers are used for the evaluation of the clustering and ordination results (see *Table 4.2.4.1*) because the tributaries are excluded from the analysis due to their very different character.

According to the three illustrating dendrograms (*Figure 4.2.4.1*), Novi Becej and Szeged (2 and 4), Tiszaug and Szolnok (6 and 7), and, two sites in the Kisköre Reservoir, Kisköre (8) and Aranyosi-sziget (9) are the most similar to each other based on their macroinvertebrate assemblages. On the other hand, a very long Tisza section representing the stretch from Titel up to Dombrád (ITR 1-21) forms one cluster containing altogether the lower 15 sites. The mentioned tree very similar pairs of sampling sites are involved in this main cluster.

A smaller individual subgroup can be recognized in this main one, containing the serial numbers of 11, 14, 13 and 15 (Tiszacsege, Tokaj, Tiszabercel and Dombrád). They are a bit separated from the others because characteristic occurrences of some macroinvertebrate taxa (i.e. mussels, snails, dragonflies).

The sharp division along the Tisza occurs after Dombrád. The upper five sampling sites of the Tisza occur separately from the lower 15 in another group. Sites 16 and 17 (Tuzsér and Aranyosapáti) stay individually on the dendrogram, but sites 18, 19 and 20 (Gergelyiugornya, Tivadar and Tiszabecs) form a well-separated cluster from all the others.



Figure 4.2.4.1 Plots of dendrogram of objects (sampling sites of River Tisza) after hierarchical clustering



Figure 4.2.4.2 The biplot of sampling sites and macroinvertebrate taxa (both are indicated by serial numbers, dotted lines are showing the numbers of the sampling sites)

The result of the ordination is very similar to the outcome of cluster analysis. Sampling sites 1-14 are sorted in two near groups (A). These are the Lower and Middle sections, respectively. The locations between Titel (ITR 1, No.1), the confluence section and Tiszabercel (ITR 20, No.14) are grouped in that group. There are three sites along a line (No.15, 16 and 17) that are signed by C. These are the intermediate sections between the Upper and middle stretches. Finally, serial numbers 18, 19 and 20 (ITR 24, 26, 27) are sorted in group B indicating the defined Upper stretch of the Tisza River characterized by the insect groups.

Summarizing the faunal results it can be demonstrated, that:

- Beside of the late autumn season, numerous characteristic macroinvertebrate groups were collected on the investigated Tisza stretch;
- Based on the faunal data it can be seen clearly that no evidence of cyanide or heavy metal pollution is detectable on the Tisza River. All of the most important taxonomic groups are present in relatively large taxon numbers along the whole river stretch. All of those taxonomic groups that were affected immediately after the cyanide pollution (Crustaceans, Ephemeropterans, Trichopterans and Dipterans) were recovered and repopulated the sections both in terms of taxa and earlier abundance.
- Crustaceans were especially damaged immediately after the spill. Nowadays all of the taxa repopulated the affected Tisza and Szamos stretches well.

# 5 Investigation of Tisa River- Fish fauna *5.1 Introduction*

An investigation of river fish fauna was initiated by the international commission for the protection of Danube river within in the frame of the Joint Danube Survey-Investigation of the Tisa river. The aim of the study was to examine whether the chemical accidents in the year 2000 caused damages to fish fauna. In addition the study was addressed to find out whether endocrine disrupting chemicals are present in the Tisa river. In order to ensure the comparability of data obtained from River Tisa with results from other European rivers a research strategy was applied which had been developed by the Hessian Agency for the Environment (HLUG) on behalf of the German Ministry for Science and Education BMBF (Stahlschmidt-Allner et al. 1997, Allner et al. 2001).

There is growing concern regarding environmental pollution causing reproductive failure in vertebrates including humans. A wide range of chemicals are suspected to act as endocrine disrupters on wild living fish. Apart from industrial organic chemicals and agrochemicals heavy metals are thought to interrupt the endocrine control of reproduction. HLUG monitoring concept is addressed to investigate anthropogenic effects on riverine fish fauna with special emphasis on reproductive functions. Results of the Hessian studies indicate an anthropogenic impact on fish reproductive functions in River Rhine tributaries with a high amount of sewage treatment plant discharges. River Tisa is thought to present a suitable area of investigation to carry out a comparative study, since the socio-geographic conditions differ of xenobiotic pollution, sewage treatment plant technology and hydrogeology. In addition the preceding chemical accident represents an anthropogenic impact on riverine fish fauna which requires adequate research activities.

The HLUG research strategy is based on the analysis of fish species diversity and abundance of species. In order to examine whether anthropogenic impairments of vertebrate reproductive health took place in the riverine environment two indicator species from different teleost taxonomic orders where investigated in detail. The basic mechanism of endocrine control of reproduction differ between perciform and cyprinid teleost. Presupposed species from either taxa belong to the fish fauna of the area under investigation, it is advisable to investigate a perciform fish as well as cyprinid species. The biomarker which are used to give a comprehensive description of the condition of the individual fish are listed in *Table 5.1*. In addition to data on species diversity, abundance and growth data of line 1-5 will provide an insight into the general condition of fish fauna of the area under investigation.

Data of line 6-8 will provide evidence of pollution causing reproductive failure of vertebrates.

Table	5.1:	Biomarker	,
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	Biomarker	Tissue	Method	Compounds proved to alter the adequate endpoints in laboratory Experiments
1	Age	Scales	Annual ring determination	
2	Growth	Fish	Weight and length	
3	Parasitiziation	Skin, gill, gut, coelomic cavity	Microscopy	Pesticides, immunotoxic compounds
4	Sex ratio	Gonads	Microscopy	Estrogenic compounds?
5	Gonadal weight	Gonads	Weigh, Gonadosomatic Index calculation, corpulenz index	TBT, Phenylurea herbicides
5 6	Gonadotropin- synthesis, neuro- endocrine control of reproduction	Pituitary	Immunhisto- chemistry	Heavy metals
7	Paradoxical Induction of yolk protein synthesis (Vitellogenin) in male fish	Blood	Electrophoresis of plasma proteins immunological identification of western blotted protein	Bisphenol A, Ethinylestradiol, Alkylphenolethoxylate, biodegradation products, endosulfan, PCB, DDT, TBT, other tinorganic
8	Intersexuality Testis ova	Gonads	Histology Immunhistology	compounds
9	Estradiol biosynthesis	Gonads	Tritiated water assay	TBT other tinorganic compounds,

Criteria to select sampling sites are agricultural impacts, degree of pollution caused by sewage treatment plant discharges, hydrogeological and industrial impacts. Basing on these criteria it was suggested to examine 6 sampling sites in the course of a 14 days sampling period.

# 5.2 Methods

During the sampling period 27<sup>th</sup> of September until the 7<sup>th</sup> of October 2002 a flood in the downstream area of Tisa River caused a disarrangement of shore habitats. Therefore it was impossible to ascertain data on species diversity and abundance which were comparable neither to not flooded River Tisa upstream sites nor to the HLUG monitoring strategy previously applied in course of the River Rhine survey. These circumstances forced a modification of the sampling plan. In order to avoid fishing in areas where the run away of the flood was still going on, we started the investigation of fish fauna with the most upstream sampling sites at Tivadar, where the flood already passed. It was necessary to limit the investigation of River Tisa from km 705 to km 334. According to the proposals of Hungarian experts finally the following sampling sites were selected (*Table 5.2*):

Sampling site	Characteristic	Indicator							
		species							
1.Tivadar	low degree of	bleak,							
	anthropogenic impact,	chub							
	upstream Szamos mouth								
2. Gergelyiugornya	Szamos mouth, where	bleak							
	supply of of cyanide and	chub							
	heavy metals took place								
3. Sajo	tributary , with a high	bleak							
	degree of industrial impact,	ide							
	no damages caused by								
	accidental pollution via								
	Szamos								
4. agro-chemical	impact of a singel	bleak							
plant	discharge	ide							
5.Tizafüred	area of Tisa lake; hydraulic	bleak							
	engineering techniques	ide							
	were applied to prevent								
	the nature reserve from								
	contamination with the								
	heavy metals and cyanide,								
	supplied via Szamos River								
	during the periods of								
	accidental pollution.								
6. Szolnok	industrial centre,	ide							
		bleak							
7. Titel	cause of the flood only spec	ies							
	diversity,- abundance and	_							
	parsitization were investigated,								

 Table 5.2: Sampling sites



# Figure 5.1: Map with sampling sites

As mentioned before, due to the flood it was impossible to investigate the impact of the eastern tributary Maros River at Szeged.

Analysis of species diversity of Titel sampling site revealed that the abundance of perciform fish in River Tisa was surprisingly low. The number of individuals collected from *Perca fluviatilis*, a predominant species in middle and west European rivers, was not sufficient for a statistical analysis of the results. This situation caused to choose the small sized cyprinid *Alburnus alburnus* (bleak) as indicator fish. This surface feeding species is thought to be present over the total stretch of the river. Additionally *Leuciscus cephalus* (chub) and *Leuciscus idus* (ide) which are the predominant species of the upstream and the downstream region respectively were investigated as indicator species for endocrine disruption. In contrast to bleak, chub and ide are related to the benthic food chain.

These modifications had the following consequences on the monitoring design:

- 1. Sex determination in cyprinid fish requires microscopically/histologically examination of gonadal tissue preparation, whereas macroscopically investigation of gonadal anatomy is sufficient to provide evidence for gender of perciform fish. Therefore the lack of suitable perciform species caused an increased expenditure of work in the course of sex ratio determination.
- 2. Gonadotropin producing pituitary cells in cyprinids are spread over the entire organ, whereas in perciformes gonadotropic cells are arranged as a compact tissue and restricted to defined areas of the gland. The application of

immunhistological methods to identify gonadotropic cells in cyprinides represent a time consuming procedure and requires sophisticated morphometric techniques. This situation forced to step down monitoring neuroendocrine control of reproduction on the pituitary level in favour of sex ratios determinations of the cyprinid indicator species monitored in course of the survey.

3. The consequences of the organisational alterations of the survey forced by the flood was, that the laboratory boat ARGUS was not available for the fish fauna investigation of the upstream sites. Fishing there was carried out by using cars and rubber dinghy (*Figure 5.2b*). Tissue collection was performed at the shore by using mobile laboratory equipment (*Figure 5.2a*). Under this working condition storage of gonadal tissue suitable for measurement of aromatase activity (in liquid nitrogen or at –80°C) was not possible and monitoring of this biomarker had to be cancelled.



Figure 5.2a. Field "laboratory"

Figure 5.2b. Electrofishing team

Fish were caught by electrofishing using strength of electric current between 3 and 6 ampere. Applying this method only individuals inhabiting the shallow water area up to 1m in depth and 1-3 m distanced from the shore were considered. Species variation and analysis of species abundance was carried out immediately. Fish of nonindicator species were put back into the river. So far available maximal 40 individuals from the indicator species were killed by cervical dislocation. Blood samples for vitellogenin determination were taken from the dislocating cut. After weighing and measuring of the fish the gonads of individuals > 4 cm were removed and weighed for gonadosomatic index calculation. An aliquot of gonadal tissue was examined microscopically directly for sex determination. The remaining gonadal tissue was fixed in Bouin-Hollandes fluid for histopathological investigation. Further an aliquot of liver tissue was fixed for immunohistological identification of vitellogenin and liver histopathology. Since gonads of fish smaller than 4 cm are not recognisable by stereomicroscopical investigation of the body cavity these specimen were fixed *in toto* for histological sex determination.

#### Vitellogenin determination

Depending on the size of the fish blood for vitellogenin determination was collected in 5-50µl steps from the dislocation cut by using Eppendorf-pipettes. Blood samples were transferred immediately into a buffer solution containing the protease inhibitor Aprotinin in order to avoid proteolysis. After centrifugation (5min 5.000 x g) the supernatant was stored at -18°C until electrophoretical separation of blood proteins by SDS-PAGE according to Allner et al. (1999).

SDS-PAGE identification of serum vitellogenin was verified by using immunological methods. Carp vitelogenien antisera according to Hennies et al. (2002) were applied for the immunological identification of serum proteins by western blot.

#### Age determination

Age determinations were carried out by scale analysis. 20-30 scales from the dorsal trunk region were taken and stored in small paper bags until analyses. To check the number of annual growth rings microscopically, epidermal residues were removed using an household dish washing detergent. Cleaned scales were fixed between two microscope slides and were examined microscopically. Age-growth classes were determined, basing on age determination of 35-40 individuals of each sampling site.

#### Sex ratio determination

Determination of sex ratio was carried out by microscopically check of aliquots of fresh gonadal tissue immediately after removal of the organ in course of tissue sampling. When the microscopically examination of unfixed unstained tissue did not provide evidence for the gender of the individual, sex was determined by histological investigation of fixed gonadal tissue. The confidence interval indicating the range of possible values of sex ratio within the population in relation to the spot check at the 95% level was calculated by using the formula:

$$dp = \frac{\sqrt{np(1-p)}}{n} * 1,96$$

n = number of samples p = frequency of one (of two) alternatives

#### 5.3. Results and discussion

#### Results of faunal investigation

In course of the survey 42 species of fish were proved to belong to the River Tisa fish fauna (*Figure 5.3*). The highest species diversity was observed in the area of Tisa mouth at Titel (24 species). These findings confirm the assumption that species diversity is mainly influenced by geographical impacts of the watershed. Assuming increasing species diversity in downstream areas of rivers as a general pattern of species distribution, the number of species found at Tisa Lake (22 species, at km 431) and Gergelyiugornya (20 species, at km 685) are surprisingly high. These findings indicate an impact of habitat structure on species diversity. Both sites provide shallow and sandy shore regions or a macrophyte stand. The comparison of species diversity between Szamos mouth area (Gergelyiugornya, 20 species, at km 685) and the upper most sampling site Tivadar (10 species, at km 705) does not provide evidence for a reduction of species diversity caused by accidental pollution in 2000 via Szamos River. The lowest species diversity (8 species) was estimated 100 km downstream from Tisa Lake at Szolnok (km 334). Further predominance of species showing a high degree of benthos orientation at this site is remarkable.

The number of fish caught at the different sampling sites decreased downstream (n=463 at Tivadar to n=142 at Szolnok). This result reflects a semi quantitative measure of fishing success and thereby of the abundance of fish at different sampling sites. Regarding the fishing success in the area of Sajo mouth it is important to emphasise that no fish were caught over a stretch of 700 m downstream the discharge of the agrochemical plant and in Sajo River from km 0,8 to 1,5.



Figure 5.3 Species frequency (to be continued)





Species	Titel	Gergelyi- uqornva	Sajo	Szolnok	Tivadar	Tisa-Lake	Tisa downstream Saio mouth
Abramis ballerus	Х						
Abramis bioerkna	Х	Х		Х		Х	Х
Abramis brama	Х	Х		Х		Х	Х
Abramis sapa	Х	Х		Х		Х	Х
Alburnoides bipunctatus		Х			Х		Х
Alburnus alburnus	Х	Х	Х	Х	Х	Х	Х
Aspius aspius	Х	Х	Х				Х
Barbus barbus		Х	Х		Х		Х
Barbus petenvi					Х		
Carassius gibelio	Х	Х	Х			Х	Х
Chondrostoma nasus		Х	Х	Х	Х		Х
Cobitis taenia			Х		Х		
Cyprinus carpio				Х		Х	
Cyprinus carpio (domest.)						Х	
Esox lucius	Х						
Gobio albipinnatus	Х	Х			Х	Х	Х
Gobio gobio					Х		
Gymnocephalus baloni	Х					Х	
Gymocephalus schrätzeri		Х				Х	
Нуро	Х						
Lepomis gibbosus	Х	Х	Х			Х	Х
Leucaspius delineatus		Х					
Leuciscus cephalus	Х	Х	Х		Х	Х	Х
Leuciscus idus	Х		Х	Х		Х	Х
Lota lota		Х					
Misgurnus fossilis							
Neogobius fluviatilis	Х					Х	
Perca fluviatilis	Х		Х			Х	Х
Perccottus glehni							
Proterorhinus marmoratus						Х	Х
Pseudorasbora parva	Х	Х	Х				
Rhodeus sericeus amarus	Х	Х			Х		
Rutilus rutilus	Х		Х	Х		Х	
Sabanejewia aurata					Х		
Sander lucioperca	Х	Х				Х	
Sander volgense						Х	
Scardinius erythrophthalmus	Х					Х	
Silurus glanis		Х				Х	
Vimba vimba	Х						
Zingel zingel	Х	Х					
Ictalurus nebulosus						Х	
Ictalurus melas							

# Table 5.3. River Tisa fish fauna

# Population structure of indicator species

# Bleak (Alburnus alburnus)

Data on species frequency show clearly that bleak was the only species present at all sampling sites. Further abundance of bleak was sufficient to enable the removal of 40 individuals / sampling site as required for a statistical analysis of the results. Relative frequency of bleak decreases downstream and varies between 79% at

Gergelyiugornya and 36,3% of the total caught at Titel. The abundance of 2+ individuals was low at Gergelyiugornya and at Tisa Lake. Significant numbers of 2 year old bleak only were proved at Szolnok and downstream Sajo mouth. Further 3+ individuals occurred at these sampling sites, exclusively (*Figure 5.4*).



Figure 5.4: Age structure of bleak population at different sampling sites

The comparison between bleak age distribution at Tivadar and Gergelyiugornya shows a higher degree of variance of body length of 0+ individuals. These results may indicate a prolonged spawning season at the area of Szamos river mouth. These data do not provide evidence that the chemical pollution in 2000 caused a significant reduction of the abundance of fish elder than one year in the area of River Szamos confluence.

The reduction of bleak population at Tisa Lake (41,4%) comprises all age classes, whereas the low abundance of bleak (39%) at Szolnok sampling site is due to the significantly reduced number of individuals originating from the 2000 spawning season and a single cohort of 0+ individuals. Data on species diversity at Szolnok do not provide evidence for the presence of predators or of food competitors of *Alburnus alburnus*. These peculiarities of bleak population structure at Szolnok area might be

indications of an anthropogenic impact on bleak reproductive success of 2 and 3 year old individuals in 2001 and a nearly complete elimination of 2000 offspring.

In the area of discharges of the agrochemical plant downstream of Sajo mouth the predominance of individuals measuring 5 cm total length among the 0+ individuals may be a hint to growth inhibition or a shift of spawning season (*Figure 5.4*).

### Chub (Leuciscus cephalus)

The second indicator species of the upper Tisa was *Leuciscus cephalus*. Bleak and chub differ regarding body growth and generation time and nutritional type. Therefor the effects of continuous as well as of accidental pollution on population structure of either species might be different.

Abundance of 0+ year old individuals at Gergelyiugornya is significantly lower than in Tivadar. This might be a hint to an impairment of reproductive success in the year 2001 of chub inhabiting the area of River Szamos confluence. Sexual maturation of female chub takes place in the 4+ individuals. Since no chub elder than 3 years were caught the question arises whether adults of this species don't live in the areas under investigation (*Figure 5.5*).

#### Ide (Leuciscus idus)

It was intended to investigate *Leuciscus idus* as the second indicator species of the downstream sampling sites. Only at Szolnok abundance of this species was sufficient for statistical analysis. At Szolnok only individuals elder than two year were caught, although the shallow shore areas under investigation, e.g. at Tisa Lake, are thought to represent suitable spawning grounds (*Figure 5.5*).





# Parasitiziation

The analysis of infection rates show that parasitic infection of fish was elevated in the area of Sajo mouth and selectively slightly increased in chub in the area of Samosz



confluence (*Figure 5.6*). Apart from fungal mycosis caused by Branchiomyces and Basidiobolus and protozoosis caused by ciliates occurring in the area of Sajo mouth, parasite spectra observed in course of the River Tisa survey did not differ significantly from parsitiziation of River Rhine fish.

Figure 5.6a. Parasitiziation of indicatorspecies

Protozoosis caused by ciliates of the genus Amiophrys, observed in the area of Sajo mouth, are due to the high affinity of this parasite to the dominant species *Alburnus* 



*alburnus* of River Tisa fish fauna. Sex specifc sensitivity of male fish from Sajo River mouth represents an interesting parallel to parasitiziation pattern of River Rhine fish. Male roach (*Rutilus rutilus*) originating from a River Rhine harbour with a high degree of tributyltin sediment pollution, also showed a significantly elevated abundance of gill parasites.

# Figure 5.6b Gender specific infection rate

### Investigation of sex ratios

#### Sex ratio of bleak

Sex ratio of bleak varied significantly between the upstream sampling site Tivadar and the area of Samosz River confluence (Gergelyiugornia). The "unpolluted" upstream site Tivadar showed a female surplus, whereas 20 km downstream a shift towards a statistically significant male surplus was observed. Apart from Tisa Lake all downstream sampling sites showed a more or less pronounced tendency to more male than female individuals.





#### Figure 5.7. Sex ratio of indicator species

numbers of chub and ide were not sufficient for statistical analysis at all sampling sites.

Confidence intervals indicate the range of possible values of the real sex ratio of the population in comparison to the spot check at 95% level.

Data on sex ratios of ide and chub did not provide evidence for a statistical significant deviation of 1:1 male : female sex distribution. The total number of 14 ide caught in the area of the Sajo mouth was not sufficient for a statistical valid analysis of sex ratio (2 male and 12 female individuals, *Figure 5.7*).

### Vitellogenesis

### Vitellogenesis in female fish

Data on number of vitellogenin producing female individuals obviously reflect the age structure of the population of the sampling sites. E.g. at the area of Sajo mouth mostly 2+ individuals were caught. Since vitellogensis in ide starts in 3+ individuals the low number of vitellogenin producing individuals primarily reflects the normal life cycle of this species. At the downstream sampling sites mostly sexual mature (4+, 5+, 6+) female ide were caught. Also the high amount of vitellogenin producing female bleak at Szolnok confirms with the analysis of age structure, showing an extremely low abundance of immature fish (*Figure 5.8*).



# Figure 5.8. Vitellogenesis in females of indicator species

Vitellogenin identification in chub created problems since a protein showing only minor differences in comparison to vitellogenin, occurred in wide varying amounts in male as well as in female chub. Immunlogical vitellogenin identification of western blotted cyprinid serum

protein provided evidence for the existence of two closely neighbouring but separate immuno-reactive bands. Since in chub the alterations of one of the two bands does not correspond neither to the gender nor to the state of reproduction, a cross-reactivity to a protein with unknown function has to be assumed (*Figure 5.9*). Therefore vitellogenin identification in this species requires additional steps of protein separation. The low number of vitellogenin producing female chub does not reflect the observed lack of mature individuals. It seems that vitellogenesis in this species took place randomly and was not related to the age and thereby to the reproductive state. These results require a critical review of the method of vitellogenin identification in this species.



Figure 5.9. Densiogramm of serum proteins from female chub Insertion: Western blot showing immunoreactive proteins in carp serum detected by vitellogenin antiserum. Two distinct proteins were detected in female (KP) serum, no vitellogenin in male carp serum (KS) (MGM: molecular weight standard)

#### Paradoxical induction of vitellogenesis in male fish

Data on vitellogenin syntheses in chub show similar features in males and females. The elevated number of vitellogenin producing males might reflect a hint to pollution with endocrine disrupting compounds.

Data on frequency of vitellogenin induction in male bleak and ide show a definite paradoxical induction of vitellogenin. downstream the agrochemical producing plant in the area of Sajo mouth abundance of vitellogenin producing males of the surface feeding species bleak was elevated while vitellogenesis of the benthivorous species ide was not affected. Downstream the city of Szolnok the more benthic species ide showed a clearly elevated number of vitellogenin producing males whereas the proportion of vitellogenin producing male bleak were only slightly elevated at this sampling site (*Figure 5.10*). The underlying basic mechanism of endocrine control of reproduction are comparable in all cyprinide teleosts. The habitat preference and the nutritional type of the species represent the decisive factor for the differential reaction of bleak and ide regarding paradoxical induction of water and suspended organic matter with xenoestrogens may have caused the species specific reaction of bleak downstream of agrochemical plant and that a sediment born pollution caused vitellogenin induction in ide at Szolnok.



# Figure 5.10. Induction of vitellogenin in males of indicator species.

#### General discussion and conclusions

The results of the investigation of River Tisa fish fauna showed, that the HLUG strategy of River Rhine monitoring is applicable to the Danubian system. The combination of faunal and ecotoxicological methods provides useful tools regarding the retrospective assessment of accidental as well as of continuous pollution of the riverine environment.

The rapid resettlement in the course of the first year after the accidental pollution confirms with the data on the compensatory capacity of the riverine environment after the Sandoz-spill in 1986.

River Rhine was contaminated by organic pollutants, therefore either accidents should only be compared regarding the compensation of lethal effects.

Since River Tisa survey was the first investigation of an accidental polluted river using a monitoring strategy comprising both population ecology as well as reproductive physiology and –health, the final evaluation of the data requires a repetition of the survey at a later time. Basing on data obtained from a single sampling it is not possible to decide whether the lack of immature fish, e.g. at Szolnok, was due to selective toxicity of accidental supplied pollutants or whether fertility of ide is reduced significantly and the "population" mainly consists of migrating individuals originating from (unpolluted?) sites.

Analysis of age structure of River Rhine cyprinids did not provide evidence for a complete elimination of distinct age classes of a single species. To our experience population structural features which are comparable to the Szolnok results are restricted to perch (*Perca fluviatilis*) population of lake habitats and thought to be related to temporary predatory pressure caused by pike-perch (*Stizostedion lucioperca*) and pike (*Esox lucius*).

Downstream the agrochemical plant and at Szolnok paradoxical induction of vitellogenesis took place. These findings indicate a local pollution with endocrine disrupting chemicals. Therefore it can not be excluded, that effects on the population level are caused by either the accidental pollution and by local pollution. The higher

compensatory capacity of the upstream sampling sites might be due to structural features of the river, e.g. the connection to neighboring unpolluted habitats provides a higher potential of resettlement.

Findings of the comparative investigation of sex ratio and induction of vitellogenesis are in accordance with results of River Rhine monitoring. Both studies do not confirm the hypothesis that induction of vitellogenin indicates a feminisation of the individual and a shift of sex ratio to a higher portion of females finally resulting in the feminisation of the population.

In view of the high water volume of River Tisa, the complete elimination of the fish fauna over a stretch of nearly 1000 m downstream the discharge of the agrochemical plant and the high amount of vitellogenin producing male bleak in the area 1000-1500 m downstream the discharge should trigger further investigations. In course of an ongoing Hessian monitoring studies comparable rates of vitellogenin induction occur in small creaks containing an extremely high amount (50-80%) of sewage treatment plant effluents or at harbour sites, where sediments are contaminated with endocrine disrupting chemicals (tinorganic compounds). A more detailed study especially of River Tisa downstream Sajo mouth seems to be useful to answer the general question whether the lower burden of endocrine disrupting chemicals accompanied by lower incidents of paradoxical induction of vitellogenesis in continental central European rivers compared to British or western European rivers is due to water quality management or to differences in hydrogeology (Purdom et al. 1994, Sole et al. 2000). A screening of organic contaminants in order to investigate the distribution and the fate of the discharged organic contaminants in relation to the effects on fish fauna is advisable to asses the risk of an irreversible contamination of drinking water resources and nature protection reserve of the region.

The investigation of *Leuciscus idus* population in the wild represented a unique opportunity to asses data of a comprehensive HLUG laboratory study with this species. The variety *Leuciscus idus melanotus* is used as a test fish in German water quality management to determine the lethal toxicity of sewage treatment plant effluents. The HLUG carried out a research project, which was addressed to modify the test procedure of the DIN 38412 L 31 in order to monitor lethal effects and reproductive toxicity simultaneously (Allner et al. 2000). Measurement of reproductive toxicity is based on vitellogenin determination in blood of golden ide after a 4-7days lasting exposure to diluted sewage treatment plant discharges. The data regarding population structure and paradoxical induction of vitellogenesis from Szolnok site confirm the results of HLUG laboratory experiments, indicating a high sensitivity of *Leuciscus idus* to endocrine disrupting compounds. Since the modified fish test using *L. idus* as a test species enables to determine the degree of lethal toxicity as well as the potential to cause chronic damages of fertility this test system might be a useful tool in water quality management of eastern Europe.

### 6. Conclusions

An international sampling expedition was carried out with the help of the HLUG (Wiesbaden, Germany) using the ARGUS ship, which had facilities for sample collection and laboratory analysis. The sampling program was carried out between 28 September and 9 October 2001 from Titel (Republic of Yugoslavia) to Tiszabecs (Hungary-Ukraine), along the 744 river km river stretch.

The concentrations of the most important inorganic nutrient substances were not found to be high in case of the Tisza River. There were only few tributaries where the extreme values were detected such as the Bega River (ammonium and nitrite nitrogen) and the Zagyva River (orthophosphate phosphorous) most probably due to permanent organic load. In case of orthophosphate phosphorous concentration the Sajó River had a little bit increased value due to the communal wastewaters of Miskolc city. Higher concentrations were found in the Zagyva River.

In the Tisza River - due to the flooding situation - the concentration of chlorophyll-a generally was not high at all, especially in the Upper Tisza stretch. The maximum value was measured at the downstream end of the Kisköre Reservoir. However, the Zagyva River with the detected 73  $\mu$ g/l chlorophyll-a concentration seems to be an outstanding water course in that respect, too. Generally it can be stated that the Tisza and its tributaries are not nutrient limiting water bodies.

Interesting data were collected for the inorganic and organic micropollutants in water, suspended solids, sediment and mussel species. The mercury, cadmium, lead, chromium, copper, nickel, zinc, aluminum, iron, manganese and arsenic total concentrations showed characteristic longitudinal profile in *water* along the Tisza: moderately low values in the upper and middle section of the river and significant increased downstream of the Maros confluence. Only a minor part of the total metal content was in dissolved form. The dominant proportion of the metals was attached to suspended particles.

The heavy metal concentrations in *suspended solids, sediment and mussel tissues* varied in a wide range. Data on *Unio tumidus, U. pictorum* and *U. crassus* are available as the results of the ITR program referring to the Tisza and its tributaries.

Data indicate that several heavy metals (copper, nickel and mainly zinc) occured at increased concentrations in suspended solids and sediments. There was an interesting mixing up/remobilization phenomenon experienced during the ITR mission concerning the mercury. The largest portion of this element was detected in the suspended solid phase. Mercury was a characteristic pollutant of the Sajó River in the past. Although the pollution has been stopped, it is still present and available in certain sediment depositions. This pollutant can be remobilized by mixing up processes during flooding.

In case of cadmium, relatively high values were measured in suspended solids, sediment and mainly in mussel species. Lead and copper generally exceed the limit values in the sediment compartment. The typical zinc concentrations in the Tisza are two times higher than the limit. Zinc was found in the mussel tissues in a considerable amount. It is clear from the diagram that larger concentrations were

detected in the lower Tisza (Yugoslavia) and in the Kisköre Reservoir. Maximum values were near to 2000 mg/kg dry weight, indicating that this compound causes serious pollution problem in the whole region.

Organic micropollutants were surveyed in water, sediment and mussels. Polar pesticides - atrazine, simazine, propazine and endosulfan were analysed in water to estimate the effect of agricultural pesticide application. From these four pesticides atrazine, simazine and propazine were found above detection level (>1 ng/l) in moderate concentrations. Remarkable (but below target value) atrazine concentrations were found in the Maros River and in the Tisza downstream of the Maros, presumably owing to the intensive surface runoff from arable land in the Maros river basin.

Selected organochlorine compounds (lindane, pp'-DDT, hexachlorobenzene, hexachlorobutadiene, pentachlorobenzene), polychlorinated biphenyls (PCBs) and polyaromatic hydrocarbons (PAHs) were analyzed in suspended solids, sediment and mussels because these pollutants tend to be accumulated in solid phase. The mean concentration levels of the above mentioned pollutants were similar in the Danube and in the Tisza during the JDS and ITR surveys. These pollutants had moderate mean concentrations in the suspended solids, sediment and mussels in both rivers. Some PCB-28 values in sediment and suspended solids exceeded target value in the tributaries Sajó and Bodrog and in the Tisa River at Tiszabercel.

The results of the algological investigations show that phytoplankton species diversity, abundancy and chlorophyll-a were relatively low from station Titel (the confluence of the Tisza River to the Danube) along the whole Tisza River. The reason of that was the flooding situation and high water level. Generally, the diatoms (Bacillariophyceae), green algae (Chlorococcales) and Euglenophyceae were the most dominating taxa in the whole investigated stretch of the Tisza and as well as in its tributaries. Green coccal and flagelate algae created second abundant group from Titel to the confluence of Bodrog. In the upper part of the Tisza (from Sajó confluence) numbers of cells of Chrysophyceae increased.

Summarizing the results of the zooplankton investigation series conducted in September - early October it is concluded in general that only 55 Rotatoria and Crustacea species lived in the Tisza and at the confluence points of its tributaries – in lower than usual values. Dominant species were however identical to previously observed ones. Occurrence of rare species became even more seldom compared to the results of previous investigations.

Individual abundance values of the animals are usually lower by one-two magnitude of orders than in previous years during the same season (late summer – early fall). No increase in individual numbers was observed due to impoundment and on downstream sections. One possible reason for this is the narrow food resources for the filter feeder zooplankton species as it is evidenced by the measured low chlorophyll a concentration values.

Summarizing the results of the macrofauna it can be demonstrated, that beside of the late autumn season, numerous characteristic macroinvertebrate groups were collected on the investigated Tisza stretch. Based on the faunal data it can be seen
clearly that no evidence of cyanide or heavy metal pollution is detectable on the Tisza River. All of the most important taxonomic groups are present in relatively large taxon numbers along the whole river stretch. All of those taxonomic groups that were affected immediately after the cyanide pollution (Crustaceans, Ephemeropterans, Trichopterans and Dipterans) were recovered and repopulated the sections both in terms of taxa and earlier abundance. Crustaceans were especially damaged immediately after the spill. Nowadays all of the taxa repopulated the affected Tisza and Szamos stretches well.

Multivariate analysis of macroinvertebrate data indicates that there are two main stretches on the investigated 744 km long Tisza River. The sampling sites on the Lower and Middle Tisza form one main group including the lower 14 sampling sites on the Yugoslavian-Hungarian stretch. The Upper Hungarian Tisza differs very much from the other section in the macroinvertebrate community. There was an intermediate section between the Upper and the Middle Tisza stretch having a transitional invertebrate fauna.

The overall conclusion of the JDS ITR sampling mission is that no detectable effects of the cyanide pollution were observed in the water, suspended solids, sediment and the biota. All of those taxonomic groups of aquatic macroinvetrebtares that were affected badly immediately after the cyanide spill had recovered successfully according to the findings of the ITR sampling program. However, the level of some inorganic and organic mictropollutants was significant in certain investigated compartments. All of the data obtained during the sampling program will be valuable and useful for the future comparison either within the Tisza River system or elsewhere in the Danube River Basin.

Concerning the results of the fish study it can be concluded that a more detailed study especially of River Tisa downstream Sajo mouth seems to be useful to answer the general question whether the lower burden of endocrine disrupting chemicals accompanied by lower incidents of paradoxical induction of vitellogenesis in continental central European rivers is due to water quality management or to differences in hydrogeology. The data regarding population structure and paradoxical induction of vitellogenesis from Szolnok site confirm the results of HLUG laboratory experiments, indicating a high sensitivity of *Leuciscus idus* to endocrine disrupting compounds. Since the modified fish test using *L. idus* as a test species enables to determine the degree of lethal toxicity as well as the potential to cause chronic damages of fertility this test system might be a useful tool in water quality management of eastern Europe.

## List of participating institutions and persons during the ITR mission

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