EVOLUTION OF THE EXPERIMENTAL PLAN IN DATA COLLECTION AND VERIFICATION TACTICS IN MODELLING OF RIVER WATER QUALITY

Y. Topalova¹, I. Ribarova², Y. Todorova¹, P. Ninov³, K. Kukurin², P. Kalinkov² Sofia University, Faculty of Biology, Sofia, Bulgaria¹ University of Architecture, Civil Engineering and Geodesy, Sofia, Bulgaria² National Institute of Meteorology and Hydrology, Sofia, Bulgaria³

ABSTRACT

Modeling is one of the basic approaches for preventive management of the water quantity and quality, as well as for the ecological sufficiency of the river. The approach for selection of parameters and data collection, specific and unique for each model, is an important element in this aspect. This data plays a critical role for the calibration and verification of models. In this paper two years of investigations of the gradual adaptation of the experimental plan of data collection as well as the adequate development according to the HSPF (EPA) model and specificity of Iskar River (Bulgaria) have been presented. The 8 – degree experimental evolution was accomplished in line with the principles of EMS, ISO-14000, WFD, ecological complexity and interdisciplinary approach. The obtained results confirmed that mathematical instruments of the model require individual experimental scenario for data collection, specific for each catchment parameter, application of variety of approaches for measurement of the real rate of the processes – opposite scaling up, physical and analogous modeling, selection of critical control points, parameters and temporal scheme.

Introduction

Nowadays, the integrated management of water resources in regional and global aspect requires the simultaneous and effective application of several multidisciplinary approaches. (1) Long term monitoring of Ql&Qn of natural and anthropogenically influenced waters according to the WFD and the rules of EMS. (2) Various types of modeling to predict the evolution of water resources. (3) Highly satisfactory verification strategies of models. (4) Adaptation of modeling tools to the catchments [6, 7, 13, 19, 20, 37, 62] (Fig. 1).

The application of the above approaches will bring about the creation of adequate models describing the highly complex water systems. In spite of the huge amount of models and information in this aspect, the existent gaps are still calling for further research. The attempt at the improvement of water management tools generated the following problems:

Data scarcity is unfortunately strongly correlated to water scarcity. This includes the data for water Ql & Qn, which not only determine waters as resources but also indicate the ecological status and sufficiency of water systems [5, 11, 14, 22, 24, 33].

Another important aspect is **river/sewer** interaction – that is delivering excellent results for river levels and sewer performance with a view to lessening of flooding events (32). One of the ways to solve these problems is by establishing various models. Yet, the creation of models generated further need to fill in certain gaps. The models require extensive knowledge on the mul-



Fig. 1. Interaction of the elements of effective River Quality and Quantity management.

tiple relationships between channel hydraulic morphometry, hydrodynamics, biology, biogeochemistry – application of the principle of **ecological complexity** [29, 34, 36, 38, 49, 56].

End user concerns pose the questions about the common time/spatial density of available data as well as the strong need for integration of biology, economics, hydrology as a part of the world integrative approaches and possibilities to realize LTER (long-term ecological research) [2, 15, 61, 64, 66].

The globalization of environmental protection requires to point out the new tendencies in this integrative processes: integration of pre-accession countries like the Republic of Bulgaria in EU together with an evaluation of potential solutions and an awareness of the WFD, integration of biology and technology, integration of mathematical models and the real ecological resources and events by means of specially designed satisfactory verification programs [21, 28, 30, 39, 40, 52, 57]. Scientists apply new generation of indicative connections and processes to describe the ecological complexity. Modelers are following the idea of simplification of rather complicated ecological events. The feeling this gap is

vitally necessary [32, 60, 62, 63].

On the other hand, the highly specific nature of ecological events poses new challenges to the application of existing models. In some cases separate critical events are important, while in other - such events play only a secondary role. In this aspect, flexibility and adaptability of the models become increasingly necessary [1, 4, 25, 31, 41]. This affects not only mathematical instruments but also verification strategy and the methods and indicators used [47, 53, 65]. The so-called perceptual model needs to be gradually approximated to the reality of water catchments in order to be potentially prepared to satisfy their ecological, economic and strategic needs [44, 48, 55].

All of the above issues were a target for extended discussions among the team of TempQsim EU project. This paper presents the realization of a part of the team ideas about the evolution of the verification strategy and data collection plan in application of HSPF model for the Ql&Qn. The hydrologic aspects have been already reported in our previous papers [40, 52]. In this paper we place an emphasis mainly on the evolution of verification instruments, as well as on the biochemical, microbiological and ecological aspects. The strategy is build up on the principles of EMS^1 [2, 21] and presents an instance for a gradual adaptation of general modeling tools to respond to the specific nature of the catchments.

Materials and Methods

Study area

The Iskar River, situated in the Western part of Bulgaria, is the longest river (368 km) which entirely flows through the territory of the country. It belongs to the Da-

¹ EMS requires the application of standardized methods (ISO-14000), selection of critical control points (CCPs), as well as gradually improving of the experimental scenario according to the Deming Cycle – "Plan-Do-Check-Action"(Dess Gregory and Lumpkin 2003).



Fig. 2. Location of upper Iskar subwatershed.

nube River Basin. The selected subcatchment begins at the river spring, ends at Iskar reservoir and covers an area of 892 km² with an average altitude of 1314 m above sea level (**Fig. 2**). Geographically Bulgaria is located in the Southern part of the temperate zone, in close proximity to the subtropical Mediterranean climatic zone, which determines the moderate continental climate. During the second half of the summer and the beginning of autumn, the climate is very often affected by Azores anticyclones, which cause long-lasting dry periods (Fig. 2).

The management and realistic forecasting of the Qn&Ql of Iskar River is a subject of social, economic, ecological and health protecting interest because it is the main source of water supply of the capital of Bulgaria – the city of Sofia. For this reason the river was nominated as the most appropriate candidate for investigation in the TempQsim EU project. More data about special dynamics has already been published [42, 52, 69].

The design of the data collection and verification strategy

Running waters were characterized by longitudinal, vertical, lateral and temporal gradients. In our strategy we rely on the following general assumptions:

1. Selection of 10 key points – stations for collection of samples (7 located on the stream and 3 located on the main tributaries) on the basis of point and diffuse sources of river pollution, water extraction, extended investigation of physico-chemical and microbiological parameters every season at high and at low water (**Fig. 3**).



Fig. 3. Scheme of a part of Iskar River with the location of sampling stations. Stations: 1 - before Beli Iskar Village; 2 - after Beli Iskar Village; 3a - Cherni Iskar River; 3 - after the confluence of Beli and Cherni Iskar River; 4a - Borovishka Bistritza River; 4 - after Samokov Town near by WWTP "Samokov"; 5 - after Dragushinovo Village; 6 - Iskar River before Palakarya River; 7a - Palakarya River; 7 - before Iskar Reservoir.

- 2. Vertical investigation of the processes water, hyporheic water, river sediments at all sample stations in stream.
- 3. Seasonal influence of main tributaries on the water and sediment quality and in the case of accidental changes of water Ql&Qn.
- 4. Selection of three CCPs on the basis of monthly obtained results for intensive investigation and situations of risk events station no. 6, no. 7a and no. 7.
- 5. Application of the physical (portrait) model a small part of the river is selected for detailed investigation of the cross parameters ripal, medial (Fig. 10a). In this case we applied the principle of opposite scale up in the modeling and selection of critical reach part of the river before the reservoir 10 times longer than the width of the river in this section. In the reach in question the special set of analyses has been realized to analyze the longitudinal and cross gradients in the river.
- 6. The flush events in the river dynamics have been monitored by means of automatic equipment and simultaneous laboratory analysis according to the time scale of water Ql&Qn changes.
- 7. The critically important processes (respiration and denitrification) in the sedi-

ments are simulated at the same time in laboratory and in situ to extract the real velocity of these processes.

In **Table** for the evolution of the experimental design the following principles have been observed. (1) Opposite scale up. (2) Simulation of the processes – analogue models (denitrification/respiration chambers). (3) Application of mini model – physical model (reach scale – a segment before the reservoir 500 m long). (4) Various combinations depending on the significance of the ecological event and geographic point. (5) Combination of indicators and indicative connections with a view to optimize the low-cost but realistic management. (6) Extraction of algorithms for risk management [2, 21].

The multi-parameter automatic equipment has been used at st. no. 7 (**Fig. 4a**). The sampling of hyporheic (sediment) water has been realized by Bou-Rouch pump [9, 10]. In all experiments the standardized methods for determining water and ground water parameters have been applied according to the ISO 14000, APHA [3]. The microbiological parameters have been measured by plate count technique according to the routine practice [43]. Functional parameters (the rate of the sediment processes) have been measured by means

TABLE

Spatial/temporal steps and contents of the verification plans. T $^{\circ}$ C – temperature of water, pH, DO – dissolved oxygen (mg/l), Saturation – oxygen saturation (%), Stream rate (m.sec⁻¹), Depth of ground water (cm); COD – chemical oxygen demand, SS – suspended solids, TMC – total microbial count (bacterial abundance), OliTB – abundance of bacteria - indicators for low organic concentration, Endo – abundance of bacteria from family *Enterobacteriaceae* - indicator for fecal pollution, important for risk assessment

Steps	Number of stations	Density of sampling	Part of River Ecosystem	Analyzed parameters
1	10 (Fig. 3) Points 1, 2, 3, 7 from	Every season and at risk situation	Water	Physicochemical – T °C, pH, DO, Saturation, Stream rate, Depth of ground water
	design		and Sediment water	Chemical – NO ₃ , NO ₂ , NH ₄ , PO ₄ , COD, SS Biological – TMC, OliTB, Enterobacteria
2	3 CCPs – st. no 6, 7a, 7 Point 4 from design	Every month and at risk situation	Water	Physicochemical – T °C, pH, DO, Saturation, Stream rate Chemical – NO ₃ , NO ₂ , NH ₄ , PO ₄ , COD, SS
3	Reach (small model of the river part 500 m long) <i>Point 5 from design</i>	3 repetitions	Water	Physicochemical – T °C, pH, DO, Saturation, Stream rate Chemical – NO ₃ , NO ₂ , NH ₄ , PO ₄ , COD, SS
4	Reach (st. no 7) Automatic Equipment Point 6 from design	Every season Every month 2 times per day at flush event	Water, sedi- ment water Water, sedi- ment water Water	Physicochemical – T °C, pH, DO, Saturation, Stream rate, Depth of ground water Chemical – NO ₃ , NO ₂ , NH ₄ , PO ₄ , COD, SS
5	Reach (st. no 7) Laboratory Simulation – (Analogous Model) (according to the modi- fied by us method of Uehlinger et al. 2002)	3 repetitions in lab, 24 hour dynamics – measu- ring every 3 hours in situ control chambers – measuring every 3 hours	Sediment and sediment water	Physicochemical – T °C, pH, DO Chemical – NO ₃ , NO ₂ , NH ₄ , PO ₄ , COD, SS Functional Parameters – Rate of respiration, Denitrification, Organic transformation



Fig. 4. Denitrification/respiration chamber (*a*) and automatic equipment (*b*) for analysis of Ql&Qn at the reach /sampling point 7/.

(a)



Fig. 5. Sampling of sediment water (a) and general view (b) of sampling point 3 (see Fig. 3).

of simulating chambers according to the modified by us method described by Uehlinger et al. [50, 58, 70].

Results and Discussion

The presentation of our results will follow the idea to illustrate the gradual evolution of the experimental plan based on the levels. (1) To receive combinations of data that will meet the requirements of HSPF model. (2) Data reflecting the real ecological situation of Iskar River [40, 52]. (3) To select the critical control points (CCPs) (such as spatial, temporal and other indicators) that plays a key role in the assessment of water quality, sediments and rate of the processes in the water ecosystem [8, 68]. $(\bar{4})$ The flexibility of this plan to be coordinated with the spatial and temporal dynamics of the river. (5) In the selected CCPs the analogous and physical models to be applied to elucidate the mechanism of functioning of the Iskar River ecosystem [12]. (6) In the development and testing of the experimental design, it was especially important to follow the principles of efficiency and effectiveness of the in situ and in lab data collection. Thus, in this paper we demonstrated the application of the "opposite scale up"¹, as well as the appli-

Biotechnol. & Biotechnol. Eq. 20/2006/3

cation of the principle of "experimental leverage" [21].

Because the purpose of this article is not to describe in detail the spatial/temporal and functional dynamics of water and sediment parameters, we will present the gradual adaptation of the plan on the basis of different case studies, as well as the application of the "opposite scaling up". Therefore, first we will start with extensive investigation in large scale and gradually focus attention to the deeper and intensive decoding of the processes in the selected CCPs.

Seasonal dynamics of water quality parameters

Initially the extended seasonal/spatial dynamics of the chemical, hydrological, microbiological parameters has been studied. Seven sampling points were chosen. They were located at all spots where changes of water quality and quantity, as well as special fluctuations of the ecological status were expected according to the previous investigation of this part of the catchments (see Fig. 3). The illustration of the spatial and seasonal dynamic of selected key parameters for the water quality is shown in **Fig. 6a, 6b, 6c**.

In the period with low waters (February, September, October) the main source of nitrogen and phosphorous pollution is town of Samokov and Wastewater Treatment Plant of town Samokov. Depending on

¹ "opposite scale up": Large scale – CCPs – reach scale – simulating of the critical processes in lab analog models – extracting the algorithms – verification of the algorithm in situ.



Fig. 6. Seasonal Dynamics of (a)-TCN (mg.l⁻¹), (b)-P-PO4 (mg.l⁻¹), (c)-TMC-wat (ln CFU.ml⁻¹).

temperature and water quantity, the most polluted spatial points were no. 4 and no. 5. In stream the water quality was improved as a whole for each season. The results showed that the other sources of pollution, respectively the sources of N and P were located before point 7. These sources and other small point sources of pollution (agriculture, fertilizers, leaves etc.) played a role for the water quality dynamics. The large amount of N and P in winter low water at point 4 corresponded with the low activity and abundance of water microbial societies [23, 26, 27, 59, 69]. By further analysis of this data and having in mind the fact that water quality is a complex function of the rate, temperature, water quantity, horizontal and vertical exchange, and the experimental plan was expanded. The seasonal dynamics of the chemical, biological and physicochemical parameters has been included in the investigations.

Seasonal dynamics of hyporheic water quality parameters

The vertical exchange of water/hyporheic water/sediments played an essential role for water quality and deeply influenced water

and sediment processes. The dynamics of chemical and microbiological parameters of hyporheic water has been studied at an extensive spatial/seasonal plan – all 7 points were subject to thorough study. A part of the data, in the form of illustration of this direction of the experimental plan, has been shown in **Fig. 7 a, b, c and d**.

In addition to the confirmation of the main sources of N, P, dynamic process of exchange between sediment water and river water, this extension of the study revealed that adsorbed microbial societies in sediments are more abundant and more active than free swimming interstitial microflora [6, 12, 69]. This data leads to the conclusion that the role of microbial biofilm is essential for the rate of the river system processes [16, 25, 27]. The rate and the mechanisms of these processes need to be examined and included in the dynamic assessment of water quality. This is a reliable ground for the prediction and management of the role of microbial societies and sediment biofilm for water quality [14]. Considering the CCPs of the exchange in vertical direction and elaborating these CCPs in



Fig. 7. Spatial Dynamics of (*a*)-TCN (mg.l⁻¹), (*b*)-P-PO₄ (mg.l⁻¹), (*c*)-TMC sediment water (ln CFU.ml⁻¹), (*d*) - TMC adsorbed bacteria (ln CFU.g⁻¹).

a temporal aspect, our experimental plan has been expanded in a horizontal direction too. The river system is completely open, and as such it strongly depends on the input of tributaries as well as on the structure and functionality of the whole all catchment area. The next step in the design was the investigation of the influence of tributaries on the water quality dynamics.

Influence of tributaries on the water quality

Three significant tributaries play a role in the dynamics of water quality and water quantity of Iskar River - Cherni Iskar, Borovishka Bistritza and Palakariya (Fig. 3). The ecological status of these tributaries differs greatly. River Cherni Iskar is highly mountainous and relatively anthropogenicaly unaffected, while the river Borovishka Bistritza collects the untreated or partially treated waters from hotels and ski tracks of the Borovetz winter resort, and the third one - Palakariya is the most polluted river due to the intensive agriculture in the region. The influence of waters coming from tributaries on Iskar River at different seasons has been studied in the next phase of our scenario. The results obtained in this phase of the experimental plan are shown in Fig. 8.

The results unmistakably confirm the important influence of the tributaries on the water quality of Iskar River. The larger amount of pollution is coming from the tributaries Borovishka Bistritza and Palakariya. The river Palakariya most

Biotechnol. & Biotechnol. Eq. 20/2006/3

strongly influences water quality in Iskar River and plays a significant role in the dynamics of water quality before Reservoir Iskar i.e. the dynamic connection of main river and tributary Palakariya is important for the description and prediction of water quality. For this reason the three CCPs (6, 7a and 7) have been selected for more detailed investigation every month.

Monthly variation of parameters at the three CCPs

The temporal dynamics (monthly and often in some critical cases) of the connection Iskar River-Palakariya has been determined. The results are presented in **Fig. 9**.

The results showed that in the periods of low water (winter low water – February; summer low water – July-September) the tributary Palakariya supplies Iskar River with organics, nitrates, phosphates and ammonia. At the high water season (May-June), the strong influence of tributary Palakariya has been confirmed, regardless of the fact that concentration gradients of biogens and organic matter in the water were lower. The deeper investigation of the functioning of the connection of Palakariya-Iskar River showed that in point no. 7 self-purification properties of Iskar River neutralize the negative effect of Palakariya and the water quality of CCPs no. 7 is improved. All this brings us to the conclusion that the ecological status, dynamics and rate of the processes in this important segment (reach) of the river determine the water quality in the reservoir as



Fig. 8. Influence of tributaries on the water quality of Iskar River (3a – Cherni Iskar, 4a – Borovishka Bistritza, 7a – Palakariya).

a main source of water supply for the capital Sofia. These conclusions became the basis for the new development of the experimental plan directed to the deeper study of the role of this reach, availability of hot spots, water, hyporheic water, sediment processes, vertical, longitudinal and cross gradients of the parameters, changes at the time of flush and risk events. Therefore, the next step in the development of the experimental plan is the selection of the part from Iskar River before the reservoir to be modeled, including point no. 7. This river segment plays the role of a physically smaller model of the whole previously investigated river section. Such kind of investigation can be called "opposite scaling up" which is opposite to the scaling up in biotechnology (lab scale, pilot scale, full scale). In our experimental plan we started from large scale examination and gradually identified the most important part of the river (500 m long) to play the role of a mini-model for detailed study of the complex relations – water quantity/water quality, availability of hot spots; flush events/water quality, role of rate of the processes in the sediments for water quality, as well as for ecological status and sufficiency of the waters and sediments etc. In our study we considered the reach as a model for detailed study. Due to the key role of the reach, the extracted relations have been discussed as essential for the whole river and especially for the part of it with key importance for the water quality of the reservoir.

Longitudinal and cross parameters at reach site

The hot spots in the reach have been determined for sampling. The purpose of this step of the experimental plan was to analyze the significance of the longitudinal and cross gradients of parameters at the section. The scheme of the set of sampling at the reach is shown in **Fig. 10a** and the results are shown in **Fig. 10b** and **10c**.

At the reach the longitudinal and cross gradients of investigated parameters have not been measured. The reason behind that

91



Fig. 9. Monthly variation of the influence of the tributary Palakariya on the water quality.

fact is the high mixing, respectively the high rate of stream. These results are basic for the selection of the section (CCP no. 7) where the automatic station was installed in order to automatically monitor the water level, water quantity and some important physicochemical parameters – pH, DO, turbidity, every 6 hours. This section CCP no. 7 plays the role of a target sampling point for determining the dynamic relation water level/water quality not only at the time of normal seasonal changes but also and especially for flush events and risk situations. We considered such kind of disturbances of the ecosystem to be very important for the prediction of the ecological sufficiency of the system as well as for the dynamic reactions for the ensuring of the high water quality.

Relationship water level/ water quality at flush event

The results showed that an accidental in-



Fig. 10. Longitudinal and cross gradients in the target area (reach) in November/December, 2003: (*a*) set of the sampling at the reach, \bigcirc - point of sampling; (*b*) longitudinal gradients in water; (*c*) cross gradients in water.



Fig. 11. (*a*) and (*b*) Relationship among water quantity (level), suspended matter (SS – soluble and unsoluble), total calculated nitrogen (TCN) and phosphorus (P-PO4) at the reach scale (sampling points no. 7).

crease of water level correlated with the increase of the biogens and suspended matter (Fig. 11a and 11b). Study of these fluctuations will create the ground for development of the modeling aiming at the prediction of specific ecological situations and will allow the application of the verified measures for the rational and safe water use.

Up to this moment, water quality, water quantity and realized processes in the water phase were in the centre of the experimental plan. Another important aspect of our scenario was the measurement and simulation of the rate of the processes in the

sediments [46].

Rate of the processes in the sediments

In our research this simulation was realized by means of analogous model in the respiration/denitrification chambers which make it possible to measure the rate of respiration and the rate of denitrification in the river sediments (**Fig. 12** and **Fig. 4**). The results are shown in the **Fig. 13a** and **13b**.

The respiration processes in sediments go through three distinct phases. The first phase includes the time from 0 to 6^{th} hour. The respiration rate is the highest and is equal to 0.55 mg O.l⁻¹.h⁻¹. The rate during the period $10^{\text{th}} - 72^{\text{nd}}$ hour is 0.11 mg O.l⁻¹.h⁻¹.



Fig. 12. (a) and (b) Rate of respiration and denitrification in the sediments at reach scale.



Fig. 13. The abundance of the Endo-bacteria in $\ln \text{CFU.m}^{-1}$ for sediment water (a) and abundance of the adsorbed Endo-bacteria in $\ln \text{CFU.g} \text{ dw}^{-1}$ sediment (b).

The oxygen level after the 72^{nd} hour is approximately 0 and the rate is negligible.

Denitrification is takes place in aerobic and in anoxy conditions. The process of denitrification is strongly dependent upon nitrate concentration, as well as upon organic matter concentration [58]. At 3.5 mg.l⁻¹ initial concentration of nitrates the rate is 0.038 mg NO₃⁻.l⁻¹.h⁻¹. At an initial nitrate concentration of 0.6 mg.l⁻¹ the rate is 0.003 mg NO₃⁻.l⁻¹.h⁻¹. These results illustrate how the analogous model tools can provide data, received by means of different experimental approaches applied by the interdisciplinary ecological team, pursuing the purposes to collect information about the ecological complexity [18, 19, 67]. At the same time, the most effective indication has been followed to obtain maximum verification information at a minimum cost, selection of the critical control points and standard methods and indicators according to the ISO and APHA. **Indicative microbial groups of bacteria** in the river sediments

Another important parameter for the potential of the sediments enriches the water with the bacteria from family *Enterobacteriaceae* is the abundance of this group [35]. The greater part of this family is patho-

Biotechnol. & Biotechnol. Eq. 20/2006/3

94

genic or conditionally pathogenic. The abundance of these bacteria and their location in various river microhabitats can be used for forecasting the changes of river water and sediment quality as well as the pollution with municipal waters in the stream. This was the argument to include the informative bacterial indicator in the experimental plan. The data illustrated dynamics of the numbers of bacteria in the sediment water and adsorbed Endo-bacteria in the sediment particles have been shown in Fig. 13a and 13b.

The results confirmed two important dependences. (1) At all the sampling stations in the stream and during all seasons the Endo-bacteria adsorbed in the sediment particles are considerably higher in number than free swimming bacteria. All this confirms that the rate of biochemical transformation processes on the sediment surface is higher, due to the concentration of substrates, microorganisms and extra-cellular enzymes [54]. (2) At the time of flush events together with the turbulence of suspended matter, sediments become the source of bacteria for the water. These processes play the role of critical factors for the changes in water quality and the rate of the transformation processes in the water, sediment water and sediments. The facts presented in this paper confirm the necessity the mathematical tools of the models to develop, to verify on the principles of ecological complexity. In parallel on the base of the mathematical instruments as well as on the base of the specificity of the river ecosystem to develop a specially constructed experimental plan for data collection [45, 51]. These data will contribute to the authentic calibration, verification of the modeling tools as well as to the realistic forecasting of the river water quality and quantity.

Conclusions

In conclusion, it is important to point out that mathematical modeling remains an

important instrument for river ecosystem management. Nevertheless, the applicability of this instrument is strongly dependent on the experimental plan of data collection, its evolution and adequate adaptation to the particular ecosystem, and to the predicted events. In this aspect the paper illustrates an example of gradual development of the data collection scenario for application of the HSPF model for the upper part of Iskar River. Here it is important to pay attention on the other demonstrated approaches for the ecological modeling – simulation of the critical processes, application of the principles of physical and analogous models in different moments on the basis of EMS, opposite scaling up (large scale, reach scale, "hot spots" (key microhabitats) processes simulation. The interdisciplinary team will continue with the further development of the experimental plan in the direction of deeper biochemical characterization of the most important microhabitats as well as their role in the formation of the general parameters of river water quality and ecological sufficiency.

Acknowledgements

This work was supported financially by the European Commission, 5th Framework program, TempQsim project, contract EVK1-CT-2002-00112 and by NSF to the Ministry of education and Science, Bulgaria – project BU–B-4. The authors express their gratitude and appreciation for the valuable assistance to Ms. Elmira Daskalova, Mr. Lyubomir Kenderov, Ms. Irina Shnaider and Mr. Dimiter Purvanov.

REFERENCES

1. Aiken G., Kaplan L.A., Weishaar J. (2002) J. Environ. Monitor., 4, 70-74.

2. Armstrong M. (1999) A handbook of management techniques. Kogan Page, London.

3. American Public Health Association, American Water Works Association, and Water Pollution Control Federation (1989) Standard methods for the examination of water and wastewater. 14th ed. American Public Health Association, American Water Works

Association, and Water Pollution Control Federation, Washington, D.C.

4. Baker M.A., Vervier P. (2004) Freshwater Biol., 49, 181-190.

5. Baron J.L., Poff N.L., Angermeier P.L., Dahm C.N., Gleick P.H., Hairston N.G., Jr., Jackson R.B., Johnston C.A., Richter B.D., Steinman A.D. (2003) Issues in Ecology, **10**, 1-16.

6. Battin T.J., Kaplan L.A., Newblond J.D., Hendricks S.P. (2003) Freshwater Biol., 48, 995-1014.

7. Benda L., Dunne T. (1997) Water Resour. Res., 33(12), 2865-2880.

8. Bobba A.G., Singh V.P., Bengtsson L. (2000) Ecol. Model., **125**, 15-49.

9. Bou C. (1974) Ann. Spéléol., 29(4), 611-619.

10. **Bou C., Rouch R.** (1967) Un nouveau champ de recherches sur la faune aquatique souterraine. C. R. Acad. Sci. Paris, **265**, 369-370.

11. Boulton A.J., Findlay S., Marmonier P., Stanley E.H., Valett H.M. (1998) Annu. Rev. Ecol. Syst., **29**, 59-81.

12. Briand E., Pringault O., Jacquet S., Torréton J.P. (2004) Limnol. Oceanogr.: Methods, 2, 406–416.

13. Bunn S.E., Davies P.M., Mosish T.D. (1999) Freshwater Biol., 41, 333-345.

14. Carvalho P., Thomaz S.M., Bini L.M. (2003) Hydrobiologia, **510**, 67–74.

15. Claret C., Boulton A.J., Dole-Olivier M.-J., Marmonier, P. (2001) Can. J. Fish. Aquat. Sci., 58, 1594–1602.

 Claret C., Marmonier P., Boissier J.-M., Fontvieille D., Blanc P. (1997) Freshwater Biol., 37, 657-670.

17. Claret C., Marmonier P., Bravard J.-P. (1998) Aquat. Sci., 60, 33-55.

18. Clement J.-C., Pinay G., Marmonier P. (2002) J. Environ. Qual., **31**, 1025–1037.

19. Dahm C.N., Baker M.A., Moore D.I., Thibault J.R. (2003) Freshwater Biol., 48, 1219-1231.

20. Dahm C.N., Grimm N.B., Marmonier P., Valett H.M., Vervier P. (1998) Freshwater Biol., 40, 427-451.

21. **Dess Gregory G. Lumpkin G.T.** (2003) Strategic management, creative competative advantages. Mc Gray – Hill, Irwin, ISBN 0-07-115106.

22. Dodds W.K., Brock J. (1998). Freshwater Biol., 39, 49-59.

23. Dodds W.K., Welch E.B. (2000) J. N. Am. Benthol. Soc., 19(1), 186–196.

24. Fauvet G., Claret C., Marmonier P. (2001) Hydrobiologia, 445, 121–131.

Biotechnol. & Biotechnol. Eq. 20/2006/3

25. Feray C., Montuelle B. (2003) Chemosphere, 50, 919-928.

26. Feris K.P., Ramsey P.W., Frazar C., Rillig M., Moore J.N., Gannon J.E., Holben W.E. (2004) Appl. Environ. Microbiol., **70**(4), 2323-2331.

27. Feris K., Ramsey P., Frazar C., Moore J.N., Gannon J.E., Holben W.E. (2003) Appl. Environ. Microbiol., 69(9), 5563-5573.

Fisher S.G., Grimm N.B., Marti E., Holmes R.M., Jones J.B., Jr. (1998) Ecosystems, 1, 19–34.
Förstner U. (2004) Lakes & Reservoirs: Research and Management, 9, 25–40.

30. Garcia-Ruiz R., Pattinson S.N., Whitton B.A. (1998) Appl. Envir. Micr., 64(7), 2533-2538.

31. Gardolinski P.C.F.C., Hanrahan G., Achterberg E.P., Gledhill M., Tappin A.D., House W.A., Worsfold P.J. (2001) Water Res., **35**(15), 3670–3678.

32. Gomi T., Sidle R.C., Richardson J.S. (2002) BioSciense, **52**(10), 905-916.

33. Grimm N.B., Gergel S.E., McDowell W.H., Boyer E.W., Dent C.L., Groffman P., Hart St.C., Harvey J., Johnston C., Mayorga E., McClain M.E., Pinay G. (2003) Oecologia, 137, 485–501.

34. Grimm N.B., Grove J.M., Pickett S.T.A., Redman C.L. (2000) BioScience, 50(7), 571-584.

35. Halda-Alija L., Hendricks S.P., Johnston T.C. (2001) Microb. Ecol., **42**, 286–294.

36. Hall R.O., Tank J.L. (2003) Limnol. Oceanogr. 48(3), 1120-1128.

37. Huettel M., Røy H., Precht E., Ehrenhauss S. (2003) Hydrobiologia, **494**, 231–236.

38. Humphries P., Baldwin D.S. (2003) Freshwater Biol., 48, 1141-1146.

39. Jansen A., Robertson A., Thompson L., Wilson A. (2004) Development and application of a method for the rapid appraisal of riparian condition. River & Riparian Land Management Technical Guideline, 4, 1-14.

40. Kalinkov P., Ribarova I., Topalova Y., Ninov P., Kukurin K. (2003) Bul. Aqua, 1(1), 35-39.

41. Kemp M.J., Dodds W.K. (2001) Biogeochemistry, 53, 125–141.

42. Kukurin K., Ribarova I., Kalinkov P., Ninov P., Topalova Y., Froebrich J. (2004) Winter low flow spatial dynamics for selected reach of Iskar River upstream. Proceedings of the International Conference of Ecology, Slynchev Brjag, Bulgaria, June, 2004.

43. **Kuznetzov S.I., Dubinina A.** (1989) Methods for determination of water microorganisms. Science, Moskow.

44. Laursen A.E., Seitzinger S.P. (2004) Fresh-

water Biol., 49, 1448-1458.

45. Malard F., Tockner K., Dole-Olivier M.-J., Ward J.V. (2002) Freshwater Biol., 47, 621-640.

46. McCutchan J.H., Jr., Saunders J.F., Pribyl A.L., Lewis W.M., Jr. (2003) Limnol. Oceanogr.: Methods, 1, 74–81.

47. Mermillod-Blondin F., Gaudet J.-P., Gerino M., Desrosiers G., Jose J., Creuze des Chatelliers M. (2004) Freshwater Biol., **49**, 895-912.

48. Morris C.E., Bardin M., Berge O., Frey-Klett P., Fromin N., Girardin H., Guinebretiere M.-H., Lebaron P., Thiery J. M., Troussellier M. (2002) Microbiol. Mol. Biol. R., **66**(4), 592-616.

49. Mulholland P.J., Fellows C.S., Tank J.L., Grimm N.B., Webster J.R., Hamilton S.K., Marti E., Ashkenas L., Bowden W.B., Dodds W.K., McDowell W.H., Paul M.J., Peterson B.J. (2001) Freshwater Biol., 46, 1503-1517.

50. Naegeli M.W. Uehlinger U. (1997) J. N. Am. Benthol. Soc., 16(4), 794-804.

51. Newbold J.D., Elwood J.W., O'Neill R.V., Van Winkle W. (1981) Can. J. Fish. Aq. Sci., 38, 860–863.

52. Ninov P., Ribarova I., Nikolaidis N., Tsoraki R., Kalinkov P., Kukurin K., Topalova J. (2004) Hydrological simulation of Iskar River subwatershed using the HSPF model. Proceedings of the ERB 2004 – Euromediterranean conference, Turin, Italy.

53. Olsen D.A., Townsend C.R. (2003) Freshwater Biol., 48, 1363-1378.

54. Palmer M.A., Covich A.P., Lake S., Biro P., Brooks J.J., Cole J., Dahm C., Gibert J., Goedkoop W., Martens K., Verhoeven J., Van de Bund W.J. (2000) BioScience, **50**(12), 1062-1075.

55. Peterson B.J., Wollheim W.M., Mulholland P.J., Webster J.R., Meyer J.L., Tank J.L., Marti E., Bowden W.B., Valett H.M., Hershey A.E., McDowell W.H., Dodds W.K., Hamilton S.K., Gregory S., Morrall D.D. (2001) Science, 292, 86-90

56. Pickett S.T.A., Cadenasso M.L. (2002) Ecosystems, 5, 1-10. 57. Pinay G., Clement J-C., Naiman R.J. (2002) Environ. Manage., **30**(4), 481–491.

58. Pusch M., Schwoerbel J. (1994) Arch. Hydrobiol., **130**(1), 35-52.

59. Ramirez A., Pringle C.M., Molina L. (2003) Freshwater Biol., 48, 88-97.

60. Reichert P., Borchardt D., Henze M., Rauch W., Shanahan P., Somlyody L., Vanrolleghem P. (2001) Water Sci. Technol., 43(5), 11-30.

61. RV Veado M.A., De Oliveira A.H., Revel G., Pinte G., Ayrault S., Toulhoat P. (2000) Water SA, 26(2) [online]. Available from http://www.wrc.org.za.

62. Shanahan P., Borchardt D., Henze M., Rauch W., Reichert P., Somlyody L., Vanrolleghem P. (2001) Water Sci. Technol., **43**(5), 1-9.

63. Schroeder R.A. (Ed.) (2003) Water-quality changes and organic-carbon characterization during recharge with recycled water at a research basin in Montebello Forebay, Los Angeles County, California, 1991–1996. Water Resources Investigations Report 03-4146, U.S. Geological Survey, Sacramento, California.

64. Sheibley R.W., Duff J.H., Jackman A.P., Triska F.J. (2003) Limnol. Oceanogr., 48(3), 1129–1140.

65. Sigua G.C., Tweedale W.A. (2003) J. Environ. Manage., 67, 363-372.

66. Stelzer R.S., Heffernan J., Likens G.E. (2003) Freshwater Biol., **48**, 1925-1937.

67. Strauss E.A., Lamberti G.A. (2002) Freshwater Biol., 47, 65-74.

68. Tank J.L., Dodds W.K. (2003) Freshwater Biol., 48, 1031-1049.

69. **Topalova Y., Todorova Y., Ribarova I. Kenderov L.** (2004) Biodiversity of microbial segment in the system "water - sediment" of the Iskar River. Proceedings of the 1-st National Conference of Ecology, Sofia, 4-5 November.

70. Uehlinger U., Naegeli M.W., Fisher S.G. (2002) W. N. Am. Natur., **62**(4), 466-473.