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Biodiversity of diatoms and macroinvertebrates in an east European lowland river, the Tudovka River (Tver Region, Russia)

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Knowledge about river ecosystems in the east European lowlands is scattered, however it is strongly needed for water management and conservation issues. The aim of the present study was to provide information on biotic key elements and their responses to major environmental gradients in a European lowland river. During the summers of 2006 and 2007, 124 diatom and 128 macroinvertebrate species were recorded in a pristine brown-water river, the Tudovka, a tributary of the Volga River. The canonical correspondence analysis showed that conductivity, pH and colour were significant environmental variables in explaining diatom data, while macroinvertebrate distribution was most related to distance from source (rkm). The eigenvalues of the first two CCA axes were significant ($p < 0.05$) for diatoms and invertebrates. However, the diatom analysis explained more of the taxonomic variation (41.7% *vs*. 34.6%). Overall, invertebrates responded more to physical factors, while diatoms depended on water chemistry, thus both components are needed for assessing river health. We suggest the use of a combination of diatom (Austrian saprobic and trophic indices, TDI and IBD) and macroinvertebrate (Austrian saprobic index, the $SPEAR_{pesticides}$ index and ITC) indices for further monitoring programmes in the Tver Region. With this study we provide important information on the riverine biocenosis in this ecosystem, as this river type is elsewhere affected by human activities.

Introduction

Biological monitoring of running waters has many advantages: in contrast to physico-chemical monitoring that provides snapshot information on a constantly changing lotic system, biotic communities integrate over a long period. A benthic algal community is affected by numerous parameters such as water chemistry, hydrology and availability of light or substrate, making algae a useful organism group for monitoring (Cox 1991, Potapova and Charles 2002, Rott *et al*. 2003). Diatoms are used frequently in monitoring European rivers (Prygiel *et al*. 1999, Round 2001, Rott *et al*. 2003) because they respond strongly to environmental changes and due to their short life cycles such changes can be identified quickly. Another advantage is that diatoms can be found throughout the year. Despite studies on diatoms in rivers (e.g. Khromov *et al*. 2002, Genkal and Kulikovsky 2005, Komulaynen 2008), lakes (e.g. Nikolaev and Harwood 2002, Mitrofanova *et al*. 2004, Meteleva and Devyatkin 2005) and palaeoecological approaches (Dorofeyuk 1978), they have rarely been used for monitoring Russian rivers (Potapova 1997). Recently a biomonitoring approach was suggested for north-western Russia to assess the influence of land-use patterns on periphyton communities (Komulaynen 2002). However, diatoms are commonly used for monitoring purposes in boreal einvironments in neighbouring countries (Vilbaste 2001, Raunio and Soininen 2007).

Macroinvertebrate biomonitoring protocols for running waters are widely used in assessment of running waters in the European Union (e.g. Birk and Hering 2004, Böhmer *et al*. 2004, Ofenböck *et al*. 2004), but less frequently in Russia (http://ibss.febras.ru/files/00006500.pdf, Morse *et al*. 2007). The governmental standards (GOST 1977, 1982) that are currently valid in the Russian Federation, prescribe (i) the use of the relative abundance of oligochaetes and (ii) the application of the index TBI (Woodiwiss 1964). Since the presence of oligochaetes is also influenced by the environment (i.e. substrate, Uzunov *et al*. 1988, Verdonschot 1989) and the TBI is considered to be insensitive (Abel 1996, Friedrich *et al*. 1996), a more accurate monitoring system is needed. Recently the Index of Trophic Completeness, based on trophic guilds, (Pavluk 2000, de Vaate and Pavluk 2004) was developed and tested in Russian and European running waters, and also in the headwaters of the Ob River monitoring activities were initiated (Beketov 2004). Recently, Litvinov *et al*. (2009) provided a review of the biota of the Volga River, however the headwaters, where this study was placed, were not considered because of lack of data.

Most streams and rivers in populated areas in the European lowland are affected by multiple stressors, such as organic pollution and morphological changes (Nijboer *et al*. 2004). Thus it is an important task to find natural streams and identify key ecological factors. In accordance with the European Water Framework Directive, reference conditions have been defined for many river types. However, data from undisturbed lowland rivers are still lacking. In the Tver region, one of the largest regions in western Russia, three main east European rivers emerge from the Valdaian hills: the Volga, the Dniepr and the Zapadnaja Dvina. The region covers over 83 000 km^2 and has a population of 1.43 million. However, the population is concentrated in the cities and thus the population density in rural areas never exceeds 5 people/km^2. Surveys in the headwaters of the Volga (Schletterer 2006) and the Western Dvina (Schletterer and Füreder 2010a) showed that large sections of these rivers are pristine (Schletterer and Füreder 2010b).

The aim of the present study was (1) to analyse the responses of the diatom and macroinvertebrate communities to major environmental gradients in a European lowland river, the Tudovka, and (2) to provide key data concerning the ecological status of a pristine lowland river.

Methods

Study area

The Tudovka River (Tver Region, Nelidovo Rayon), a right tributary of the Volga River, was selected for a monitoring programme, because its catchment is largely protected and has only few anthropogenic activities. With its paludified catchment it is a typical river in this region (Zhenikhov *et al*. 2007). The river is located in the ecoregion eastern lowlands (Illies 1978) and in the bioregion Kola–Karelian & Eastern European Forest (Krever *et al*. 1994). The headwaters of the Tudovka are located in the transition area of the Central Forest State Nature Biosphere Reserve, which was established in 1931 to protect "typical forest associations and animals of the central forest region" (Puzachenko *et al*. 2007). Since 1985 the area has been included in the International Network of Biosphere Reserves (Beltrán and Delbaere 1999, UNESCO 2006). In line with the European Water Framework Directive, the Tudovka may be categorized as type 12 ("brownwater river") (Pottgiesser and Sommerhäuser 2004).

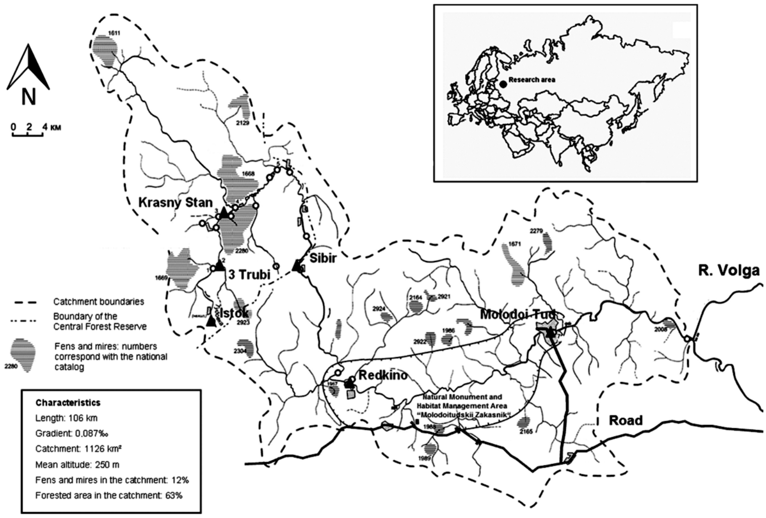

Fig. 1. Catchment of the Tudovka River, a right-hand tributary of the Volga River in the Tver Region (modified from Zhenikhov *et al*. 2007).

Sampling and species identification

Simultaneous sampling of diatoms and benthic invertebrates was conducted twice at six stations (Fig. 1), in the summers of 2006 and 2007. The Russian project partners measured conductivity, pH and temperature *in situ* while the parameter colour (chromaticity) was measured with the photo colorimeter FEK-56M with a chromium-kobalt scale in the laboratory of the Tver State Technical University (Table 1). Benthic algae (Bacillariophyceae) were sampled by brushing at least 15 stones. Where a location was dominated by fine sediments (e.g. mud or sand) the uppermost layer of the sediment (2–3 mm) was taken for analysis. The material was stored in ethanol (50%) and prepared in the laboratory using the H_2O_2 method (protocol according to Kingston 1985 as cited in Schiedele 1987). For SEM investigations the material was washed with distilled water and heated with concentrated acetic acid (2–3 min), then 1–2 ml concentrated sulphuric acid was added and the sample was heated for 15 min. Afterwards the sample was rinsed twice with 96% ethanol, then again 3 times with distilled water, before sputter-coating with gold-palladium. Determination and counting of the diatoms was carried out under a light microscope (1000×, oil immersion), using keys of Krammer and Lange-Bertalot (1986–2004), Prygiel and Coste (2000) and Kelly *et al*. (2005). On each mount 300–500 valves were counted. Further

Table 1. Selected physico-chemical parameters from the sampling sites (V. V. Kuzovlev pers. comm.).

	Sample number	Date	Temperature (°C)	pH	Conductivity (μS cm^{-1})	Chromaticity (°)
Istok	1	17 Aug. 2006	14.5	7.43	283	37
	6	22 Aug. 2007	15.5	7.29	249	47
3Trubi	2	16 Aug. 2006	19.2	7.05	196	230
	7	20 Aug. 2007	18.7	6.74	207	170
Krasny Stan	3	16 Aug. 2006	18.5	7.15	172	178
	8	20 Aug. 2007	18.0	7.02	162	230
Sibir	4	17 Aug. 2006	21.0	8.10	263	74
	9	22 Aug. 2007	21.2	8.26	289	83
Redkino		n.a.	n.a.	n.a.	n.a.	n.a.
	10	19 Aug. 2007	19.0	8.36	300	67
Molodoi Tud	5	15 Aug. 2006	21.0	8.38	332	39
	11	19 Aug. 2007	19.0	8.20	322	46

analyses were conducted with a scanning electron microscope (SEM: Philips XL 20) in order to recheck the determination of some species.

Benthic invertebrates were also collected during summer low-flow period in 2006 and 2007 along the course of the Tudovka. The samples were taken using a modified MHS method (Hering *et al*. 2003). A standard frame-net (15 × 15 cm, 500 μm mesh size) was used and according to the MHS method, all available mesohabitats were sampled. As the substrate diversity is quite small in the lowland, we sampled 10 squares according to percentages of mesohabitats (e.g. 60% lithal and 40% psammal = six samples from lithal and four samples from sand), in total 2250 cm^2. Afterwards, the material was rinsed through a 500 μm net and the invertebrates were preserved with ethanol (95%) and presorted into taxonomic groups. Determination of most taxa was carried out to species level using suitable keys (e.g. Nilsson 2005, Tsalolikhin 1994–2004), Chironomidae and Oligochaeta were identified only to family and class.

Data analysis

To estimate the maximum amount of variation in the species data and for describing patterns of species compositions and distribution of invertebrates and diatoms a detrended correspondence analysis (DCA; Hill and Gauch 1980) was performed. Species data were log-transformed and detrend by segments was used in the DCA analyses. To relate community changes to environmental gradients, a canonical correspondence analysis (CCA; ter Braak 1986, ter Braak and Verdonschot 1995) based on inter-sample distances was used. Since the longest gradient detected by DCA was not smaller than three, we applied CCA to our data. This is a direct gradient ordination method, which is suitable for biological data with unimodal responses to environmental data and a lot of absences (zeros). In total 121 diatom taxa and 128 macroinvertebrate taxa were included in the analysis. The diatom data was transformed to relative abundance. Due to their skewed distributions, all environmental variables were log-transformed before the ordination. All ordinations (DCA, CCA) were performed with the programme Canoco for Windows 4.5 (ter Braak and Smilauer 2002).

The following biotic indices were calculated, using diatom and macroinvertebrate data. Taxa Richness (TR) was assessed as overall number of taxa recorded from a particular sampling site. Shannon's diversity index (H') was calculated as follows (Shannon 1948):

$$H' = -\sum p_i \ln p_i$$

where p_i is the fraction of individuals belonging to the species i. Evenness (E) ranges from zero to one and provides information on the community structure, i.e. the closer the value to one, the greater the similarity among species abundances. Evenness (E) was calculated as follows:

$$E = H'/\ln N$$

where N is the number of species.

The following diatom indices were calculated: Generic Diatom Index (GDI, Coste and Ayphassorho 1991), Biological Diatom Index (IBD; Lenoir and Coste 1996), Trophic Diatom Index (TDI; Kelly and Whitton 1995, Kelly *et al*. 2008) and Austrian trophic and saprobic indices (Austria-T and Austria-S, respectively; Rott *et al*. 1997, Rott *et al*. 1999). The Austrian trophic and saprobic indices were calculated using the EcoProf 3.0 software (Schmidt-Kloiber and Vogl 2007). The other indices were calculated with MSExcel. GDI has a maximum value of 5, which indicates pristine water. For better comparability with IBD, GDI was converted to a scale from 0 to 20, where 20 represents pristine water. The software package "Omnidia" converts the indices (Lecointe *et al*. 1993) automatically. We used the formula:

$$\text{Index}_{\text{Transformed}} = (\text{Index}/0.05) \times 0.2.$$

Different indices were applied for the zoobenthos. The saprobic index (Moog 2002) is sensitive to organic pollution, the SPEAR$_{\text{pesticides}}$ index is a pesticide-specific monitoring tool (Beketov *et al*. 2009) and the index of trophic completeness (ITC) reflects the trophic structure of the community (Pavluk 2000, de Vaate and Pavluk 2004). Calculation of the saprobic index was carried out using the data from the Fauna Aquatica Austriaca (Moog 2002) with the Ecoprof 3.0 programme (Softwarehaus Graf and Partnes; Schmidt-Kloiber and Vogl 2007). The $SPEAR_{pesticides}$ was calculated according to Beketov *et al*. (2009) using the programme SPEAR Calculator (UFZ, Leipzig, Germany) that is freely available on the internet (http://www.systemecology.eu/SPEAR/). The boundaries of this index for water bodies of the Tver region were recently defined as: High ≥ 0.44 > Good ≥ 0.30 > Moderate ≥ 0.22 > Poor ≥ 0.11 > Bad (Schletterer *et al*. 2010c). In the same study, the boundaries for the saprobic index were defined as: High ≤ 2.18 < Good ≤ 2.51 < Moderate ≤ 2.81 < Poor ≤ 3.27 < Bad, whereas, for running waters with a naturally high degree of organic matter, the correction factor 0.25 is to be added to adjust the Saprobic basic status and consequently the other boundaries (Schletterer *et al*. 2010c). ITC was calculated with the MaTros programme (Pavluk and Bratkovskaya 2002).

Results

Diatom and macroinvertebrate communities

We identified 116 Pennales and 5 Centrales species and varieties. The Shannon diversity index for the diatom communities ranged from 1.34 to 3.38 and in a single sample numbers of taxa varied from 30 (Sibir) to 54 (Istok). In total, 128 macroinvertebrate species were identified. The Shannon diversity index ranged from 1.05 to 3.01 and the taxa numbers varied from 12 (Istok) to 36 (Molodoi Tud) per sample (Fig. 2).

The DCA analysis for diatom communities indicated clear differences between the sampled locations. The eigenvalues of the first two axes for diatom DCA were 0.469 and 0.245 and together accounted for 29.69% of the cumulative variance (total inertia = 2.404). At the source the diatom community was dominated by *Achnanthes subatomoides*, *A. bioretii*, *A. minutissima*, *Stauroneis terricola* and *Sellaphora pupula*. The middle reaches (3Trubi, Krasny Stan) were dominated by *Nitzschia palea*, *Navicula cryptotenella*, *N. radiosa* and *Achnanthes lanceolata*. Stations 3Trubi and Krasny Stan that are mainly affected by the high degree of mires in their catchment had rather similar diatom taxa. In lower reaches (Sibir, Redkino, Molodoi Tud) *Achnanthes minutissima* is predominant and a relative high abundance of *Cocconeis pediculus* indicate the presence of partly dense macrophyte stands.

The eigenvalues for the macroinvertebrate DCA were 0.700 for the first axis and 0.374 for the second axis explaining 17.98% and 9.60% of variance (total inertia = 3.894). The analysis separated the sampling stations according to longitudinal patterns similar as for the diatoms. At the Source the mayfly *Baetis tricolor*, the stonefly *Diura bicaudata*, the caddisflies *Limnephilus rhombicus*, *Oecismus* cf *monedula* and the mussel *Pisidium* sp. were important faunal components; also the cleft feeded mayfly *Metretopus alter* was present. The Middle reaches (3Trubi and Krasny Stan) were characterised by *Paraleptophlebia* cf *cincta*, *Sialis morio*, *Erpobdella octoculata* and a diverse Odonata fauna. Beaver dams caused slow-flowing and stagnant conditions at the location 3Trubi, which supported lentic and lotic species. Due to this habitat situation there is a high abundance of filter feeders, e.g. Simuliidae (*Simulium argyreatum*, *S. reptans* var. *galeratum*, *S. morsitans*) form a major part of the community in spring (M. Schletterer unpubl. data) and some species are still present in summer. The record of the filter feeding mayfly *Arthroplea congener* is typical for this habitat (river-ponds: stagnant river sections caused by beaver dams), however we found them not within the *Carex* belt (Studemann *et al*. 1987), but only in stands of water lilies. The community at Krasny Stan was characterised by the mayfly *Ephemera danica*, the caddisfly *Molanna angustata* and the mussels *Valvata piscinalis* and *Pisidium*. About 2 km upstream, near the mouth of the Khmelevki creek, in the roughly 2-m deep Tudovka, on a sandy substrate a dense population of the freshwater pearl mussel (*Margaritifera margaritifera*) was present. A population of the same species was also found in the Serioga River (Tver region, Toropezkii rayon) (M. Schletterer unpubl. data). Strong current and stony bottom were the main drivers for the community at Sibir, thus the reophil mayfly *Heptagenia sulphurea* and also *Potamanthus luteus* were present. Caddisflies

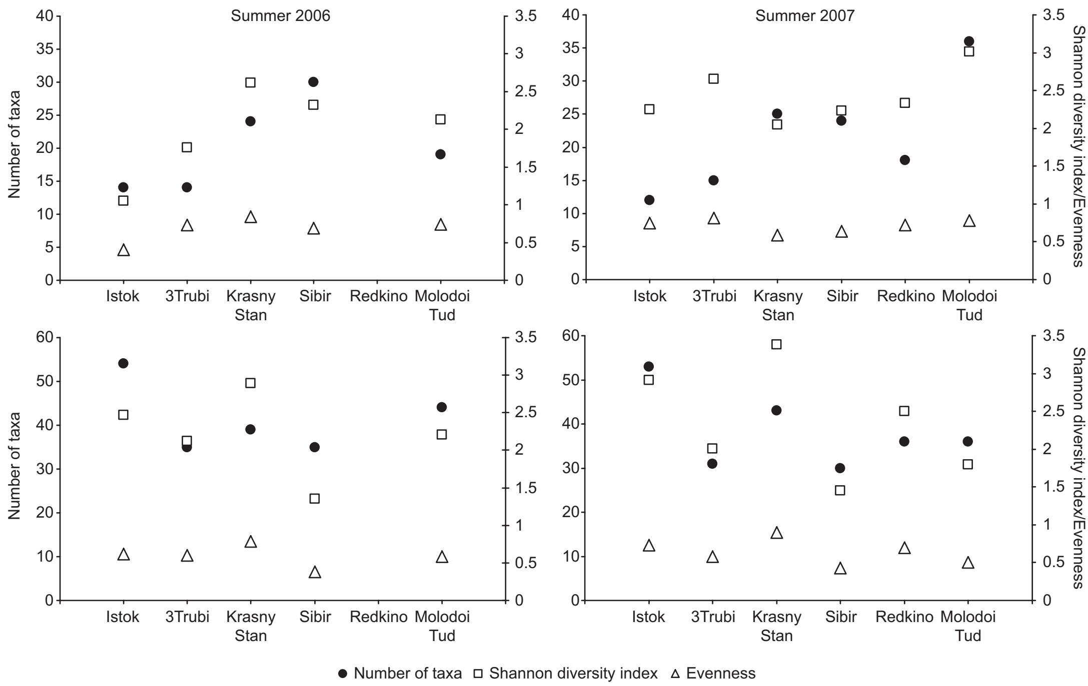

Fig. 2. Macroinvertebrate (top two panels) and diatom (bottom two panels) richness, Shannon diversity index and evenness at the six sites along the Tudovka River in summer 2006 and 2007.

(*Hydropsyche pellucidula*) and stoneflies (*Leuctra fusca*) were very abundant and also the water beetles Elmidae and Haliplidae were important components of the community. At Redkino, *Dytiscus marginalis*, *Baetis tracheatus*, *B. niger*, *Centroptilum luteolum*, *Calopteryx virgo*, *Hirudo medicinalis* were typical elements. In the adjacent floodplain, but also along the margins of the river, *Ranatra linearis* was an important predator. The lowermost station, Molodoi Tud, was dominated by hydropsychid caddiesflies (*Hydropsyche angustipennis*, *H. pellucidula*); the mayfly *Ephemerella ignata* and the water bug *Aphelocheirus aestivalis* were also common. Also *Piscicola geometra*, *Atherix marginata* and *Crunoecia irrorata* were typical at this station. At Redkino and Molodoi Tud, the stonefly *Amphinemura sulcicollis*, a typical epipotamal component, was present. Chironomidae and Oligochaeta were present at all locations.

The eigenvalues of the first two CCA axes (0.704 and 0.623, respectively; total inertia = 3.180) for diatoms (Fig. 3) were both significant ($p < 0.05$, Monte Carlo permutation). In sum, 41.73% of the total variance in the diatom communities could be explained by the first two axes. The high diatom–environment correlations for CCA axis one (0.991) and axis two (0.988), indicate a strong relation between diatoms and environmental variables. Axis one was primarily related to conductivity and pH, separating the sites from the lower course (Sibir, Redkino, Molodoi Tud) from the others. Axis two was mainly related to depth and colour. The diatom community showed a strong longitudinal zonation, which is mainly explained by conductivity, pH and colour.

Also for macroinvertebrates the eigenvalues of the first two CCA axes (0.761 and 0.542, respectively; total inertia = 3.768; Fig. 4) were both significant ($p < 0.05$, Monte Carlo permutation). The first two axes captured 34.58% of the total variance in the benthic faunal communities. High species-environment correlations for first two axes (0.987 and 0.948, respectively) indicate that the community structure is highly related to environmental factors. The first axis primarily separated the upper from the lower sections, depending mainly on the distance from source (rkm). The second axis is a gradient of humus

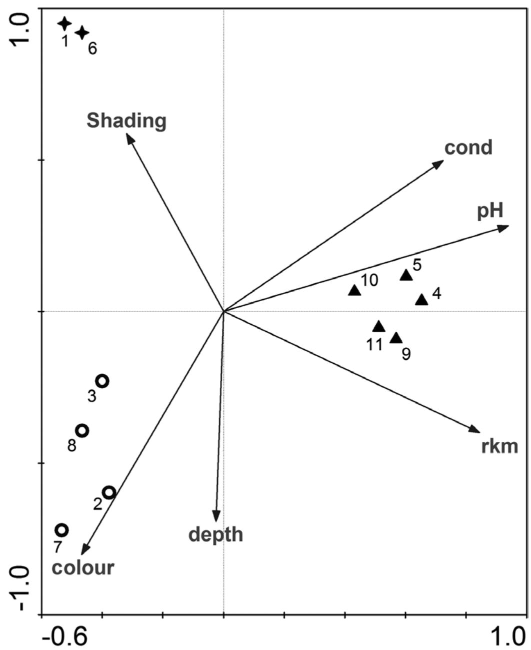

Fig. 3. CCA for diatoms from 11 samples (stars = upper course, triangles = middle course, circles = lower course of the river); the samples were taken from the same sites, but in different years.

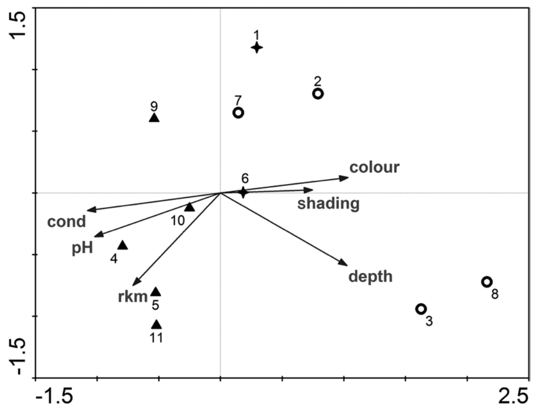

Fig. 4. CCA for macroinvertebrates at 11 samples (stars = upper course, triangles = middle course, circles = lower course of the river); the samples were taken from the same sites, but in different years.

concentration, mainly determined by conductivity, pH, shading and colour. Overall, the macroinvertebrate species distribution was most affected by the factor distance from source (rkm), but also conductivity, pH, shading and colour.

Water quality assessment

The Austrian saprobic index (Rott *et al.* 1997) characterised the stations Istok, Sibir, Redskino and Molodoi Tud as low- to moderately polluted (water quality classes I–II and II). The stations 3Trubi and Krasny Stan were classified as critically to strongly polluted (water quality classes II–III and III). The Austrian trophic index (Rott *et al.* 1999) classified the investigated river as eutrophic, which would indicate an average phosphorus level of 30–100 μg l^{-1}. The stations 3Trubi and Krasny Stan were classified eu-polytrophic and polytrophic, respectively. Similar results were obtained from TDI, IBD and GDI: TDI ranged from 30 (very low nutrients) up to 62 (very high nutrients) and indicated that 3Trubi (62), Krasny Stan (52) and Sibir (52) were the richest stations. TDI at the other stations was on average 38.7. IBD indicated high quality (oligotrophy) for the stations Istok and Molodoi Tud, the other stations having good to moderate quality (oligo-mesotrophy). GDI showed the same trend, but its values indicate worse quality in question than the values at the species level (Table 2).

According to the macrozoobenthos communities, most of the sites could be characterized as β-mesosaprobic (class II), only 3Trubi was classified as worse (β-mesosaprobic to α-mesosaprobic; class II–III). This corresponds with the regional saprobic ground status of 2.18 (Schletterer *et al.* 2010c) and thus water quality according to the Austrian saprobic index at the locations Istok, Krasny Stan, Sibir and Molodoi Tud was classified as high and at the locations 3Trubi and Redkino as good. $SPEAR_{pesticides}$ characterised water quality at all sites, except for Redkino, as high or good. In 2006, at the source (0.54), at Molodoi Tud (0.52) and at the location Krasny Stan in both years (0.50 and 0.45, respectively) water quality was high, while at the other stations, except for Redkino, it was good, with values ranging from 0.32 to 0.42. The station Redkino was classified poor (0.19), likely due to predominant substrate type (pelal) in the sampling strech. The ITC revealed high (Krasny Stan, Sibir, Redkino, Molodoi Tud) and good quality (Istok, 3Trubi) of the investigated river system (Table 2).

Discussion

Our study showed a strong influence of chemical (pH) as well as physical (colour) parameters on the diatom community. In general diatoms are known to be more indicative of hydrochemical stressors rather than changes in riverine morphology (Triest *et al*. 2001). We showed that the distance from source (rkm), together with the hydrochemical regime (pH, conductivity), was the main regulation factor for the macroinvertebrate community. Similar was also reported e.g. from northern and southern Finland (Paavola *et al*. 2000, Soininen and Könönen 2004). The distance from source integrates a complex pool of factors, e.g. river size, hydrology, light availability (shading). Since running waters are rapidly changing environments providing numerous habitat templates (Townsend and Hildrew 1994), including different biota that react to environmental changes in multiple ways, combined use of diatoms and macroinvertebrates is a promising approach for surface water monitoring.

We calculated different common European diatom indices using our data primary to estimate water quality, but also to test their suitability for monitoring programmes in the Tver Region. It has to be considered that diatom indices were developed for a particular region and that they may not be suitable for the assessment of biological integrity of running waters in other regions (Pipp 2002). The applied indices revealed similar results, thus we suggest using a combination of the Austrian saprobic and trophic indices, TDI and IBD in western Russia. The Austrian saprobic and trophic indices seem suitable for the research area, since most of the species recorded therein are included (more than 85% at most stations). The stations classified as poor (3Trubi, Krasny Stan) are surrounded by mires (about 45% of the catchment area down to the location), which was also indicated by water colour. These conditions are caused mostly by natural processes in the catchment resulting in partly oxygen-poor microhabitats at local levels. The cosmopolite species *Nitzschia palea* is known to occur in α-mesosaprobic to polysaprobic conditions and is described as one of the most pollution-resistant diatoms (Schiefele 1991, Komulaynen 2004). We found this species to be common at two sites in the Tudovka that are surrounded and highly influenced by mires. It is known from Finland that e.g. *Navicula minima*, a species tolerant to heavy organic pollution,

Table 2. Water-quality related indices based on diatoms and zoobenthos, respectively.

	Istok	3Trubi	Krasny Stan	Sibir	Redkino	Molodoi Tud
Diatom indices						
Austria-S 2006	1.40	2.89	2.12	1.96		1.78
Austria-S 2007	1.44	1.95	1.77	1.89	1.79	1.89
Austria-T 2006	2.35	3.14	2.62	2.34		2.21
Austria-T 2007	2.69	2.87	2.69	2.13	2.46	2.27
TDI 2006	30.58	56.29	50.42	52.75		36.98
TDI 2007	41.69	62.01	52.43	43.00	36.06	48.59
IBD 2006	17.4	8.2	11.2	16.2		17.4
IBD 2007	17.4	13.8	14.4	17.7	16.6	16.8
GDI 2006[1]	16.74 (4.19)	8.65 (2.16)	11.27 (2.82)	15.49 (3.87)		16.08 (4.02)
GDI 2007[1]	15.98 (3.99)	11.03 (2.76)	13.63 (3.41)	16.41 (4.10)	15.98 (4.00)	15.85 (3.96)
Macrozoobenthos indices						
Saprobic index 2006	2.00	2.49	2.14	1.84		2.09
Saprobic index 2007	1.42	2.31	1.85	1.97	2.24	1.99
$SPEAR_{pesticides}$ 2006	0.54	0.38	0.50	0.34		0.52
$SPEAR_{pesticides}$ 2007	0.42	0.32	0.45	0.33	0.19	0.35
ITC[2]	10 (11 + 12)	10 (11 + 12)	11 (12)	11 (12)	11 (11)	12 (–)

[1] converted to the scale 0–20; original values in parentheses.
[2] data from 2006 and 2007 pooled; for station Redkino only data from 2007 was available, number of trophic guilds present (the missing ones are in parentheses).

can be quite dominant at sites draining bogs and wetlands although the other community members are indicators of oligotrophic conditions (J. Soininen pers. comm.). Thus the observed dominance of *N. palea* at natural mire-sites caused the contradicting indication by the benthic communities. This also underlines that the application of these indices in mire-dominated catchments is limited and needs improvement.

The main advantage of macroinvertebrates in monitoring programmes is their reaction to different stressors like organic pollution (Moog 2002), organic toxicants in general (Beketov and Liess 2008) and pesticides in particular (Beketov *et al*. 2009) as well as to the river habitat structure (Laasonen *et al*. 1998, Muotka *et al*. 2002). Interpreting the results of macroinvertebrate surveys, it is also important to take into account functional components of the community (e.g. functional feeding types), which were assessed here using ITC (Pavluk 2000). Recently, it was shown that pristine sites in the upper reaches of the Volga River and its main tributaries are β-mesosaprobic (Schletterer *et al*. 2010c), which was confirmed by the present investigation. The conclusion drawn from $\mathrm{SPEAR}_{\mathrm{pesticides}}$ were expected, since there are only minor anthropogenic activities in the catchments. However, at Redkino water quality was classified as poor, but at Sibir and Molodoi Tud (26 km upstream, respectively 30 km downstream) as good and high, respectively. We can only explain this by the sampling method: at Redkino the river is not wadeable, therefore the multi-habitat sampling was carried out at the edges of the river where pelal (mud) substrate is common. Although sandy substrates were sampled, the main proportion of the material was from pelal, most likely resulting in classifying water quality as poor. In a previous study samples were taken from different microhabitats causing great differences in indices (e.g. $\mathrm{SPEAR}_{\mathrm{pesticides}}$ for pelal = 0.09 and for lithal = 0.43). Considering this effect, a multi-habitat-sampling method has been recommended for $\mathrm{SPEAR}_{\mathrm{pesticides}}$ (Schletterer *et al*. 2010c). The applicability of this method is however limited at non-wadeable locations, therefore, further investigation of the behaviour of this index in different microhabitats is needed.

Several faunistic key elements (e.g. *Metretopus alter*, *Arthroplea congener* and *Margaritifera margaritifera*) indicate a high environmental value of the investigated natural lowland river at the European level. The occurrence of the freshwater pearl mussel *Margaritifera margaritifera*, which is among the most endangered freshwater mussels in the world (Machordom *et al*. 2003) is especially important, since the mussel is generally supposed to live in clean salmon rivers with low mineralization at depths between 0.5 and 2 m with low turbidity (Ziuganov *et al*. 1994). However, Cosgrove and Harvey (2004) already discovered a *M. margaritifera* population in a peatland drainage in Scotland, which is considered the first record of this species in a fen habitat. Earlier this species was recorded in fen habitats e.g. in Finland in slightly acidic waterbodies that are rich in huminic acids (Lahermo *et al*. 1995). The record of two vital populations in the Valdaian hills (Tver Region, Russia) shows that a meandering brown water river may also provide suitable habitats for the freshwater pearl mussel and underlines the integrity of the investigated river system.

Conclusions

In contrast to most lowland rivers in Europe, which are impacted to a variable degree, the largest European river, the Volga, and its tributaries have remained uncontaminated in its headwaters (Schletterer 2006). The present study provided the first inventory of diatoms and macroinvertebrates in the pristine lowland river Tudovka and tested some indices in order to assess water quality and ecosystem integrity. While in Europe, diatoms and macroinvertebrates are widely used for water quality monitoring (Hering *et al*. 2003, and references herein), to date there have been no attempts to use these parameters in the Tver region. This study showed the performance of different indices under natural conditions and provided a unique dataset, since in Europe lowland rivers are highly endangered systems (Fochetti and Tierno de Figueroa 2006, Nooij *et al*. 2006, Horsák *et al*. 2009). In this regard, we suggest the use of a combination of some diatom (the Austrian saprobic and trophic indices, TDI and IBD) and macroinvertebrate indices (the Austrian saprobic index, $\mathrm{SPEAR}_{\mathrm{pesticides}}$ and ITC) in further monitoring programmes in the Tver Region and also to include this into the regional legislation.

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