

**Quantitative analysis of the macroinvertebrate community in the river
Temenica (SE Slovenia)**

Kvantitativna analiza združbe makroinvertebratov v reki Temenici (JV Slovenia)

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Abstract. Macroinvertebrate community of the river Temenica was investigated in order to assess the ecological quality of the stream. Different approaches were used in order to compare their usefulness. Surber sampler methodology was used and all together 16 quantitative samples were taken at four stream reaches every three months from October 2003 until July 2004. Saprobic and diversity indices showed severe deterioration of water quality longitudinally and a strong negative influence of the town Trebnje on the ecological state of the river Temenica. Multivariate methods DCA and CCA confirmed those results, but also showed greater differences in community structure at sampling site 1 compared to sites 2 and 3 which was not detected using the indices.

Key words: macroinvertebrates, water quality assesment, saprobic index, diversity indices, DCA, CCA

Izleček. Za oceno ekološke kakovosti reke Temenice smo raziskovali združbo makroinvertebratov. Uporabili smo različne pristope, da bi primerjali njihovo uporabnost. Uporabljena je bila metodologija Surberjevega vzorčevalnika, vzorčili smo na štirih vzorčnih mestih vsake tri mesece od oktobra 2004 do julija 2004, skupno smo pobrali 16 kvantitativnih vzorcev. Saprobni in diverzitetni indeksi so pokazali resno poslabšanje kakovosti vode po toku navzdol in močan negativen vpliv mesta Trebnje na ekološko stanje reke Temenice. Multivariatni metodi DCA in CCA sta potrdili te rezultate, pokazali pa sta tudi večje razlike v strukturi združbe na vzorčnem mestu 1 v primerjavi z mestoma 2 in 3, ki jih z uporabo indeksov nismo zaznali.

Ključne besede: makroinvertebrati, ocena kakovosti vode, saprobni indeks, diverzitetni indeksi, DCA, CCA

Introduction

Macroinvertebrates are extensively used in water quality assessment as they are considered good indicators of environmental pollution. There are various methods for assessing water quality which have different conceptual basis and may therefore provide different information about the aquatic environment. Many authors have compared different methods of summarising responses to pollution (CAO et al. 1996, ROSSARO & PIETRANGELO 1993). Water quality indices have been developed as a routine technique for water monitoring. Saprobic index is often used as a measure of organic pollution (URBANIČ 2004) and is also in use by the Slovenian Ministry of the Environment for biomonitoring of surface waters. Diversity indices are known to be inaccurate at headwater reaches and were also reported to be insensitive to slight and moderate pollution (CAO et al. 1996). However, they can still be used as a complementary method.

Multivariate methods are increasingly used in biological monitoring of water quality (CAO et al. 1996, ROSSARO & PIETRANGELO 1993). Their main advantage is that they can detect more subtle changes in community structure than the indices. They can also provide information on the responses of taxa to different environmental variables.

The aim of this study was to assess the water quality of the stream Temenica using different methods (saprobic index, different diversity indices and multivariate methods DCA and CCA) and to compare the effectiveness of those methods.

Description of study sites

Our study area was the first part of the disappearing stream Temenica in the south-eastern part of Slovenia. Its length is 25 km and the catchment area comprises 91 km².

The upper part of the stream (approx. 5 km) lies in a forested area with little human impact whereas the lowland part with meandering watercourse runs through cultivated land and by the town Trebnje, which is the largest settlement in the area. Elevations range from 560 m at the source to 260 m at the sinking point. Temperatures fluctuate between 2,2–3 °C (winter) and 14,2–18,2 °C (summer). Stream pH lies within the range 8,1–8,7 and conductivity between 504 and 604 µScm⁻¹. Sampling site 1 was established in the headwaters, site 2 at the beginning of lowland watercourse, and sites 3 and 4 upstream and downstream from Trebnje.

Materials and methods

Macroinvertebrates were sampled on four occasions between October 2003 and July 2004 with 3 month-intervals. At each sampling site 6 random sampling units were taken with a Surber sampler (500 cm² sapling area and 0,5 mm mesh size). Several physical and chemical characteristics (Table 1) were measured at the same time.

Table 1: Morphometric and physical characteristics of sampling sites on the river Temenica.
Tabela 1: Morfometrične in fizikalne značilnosti vzorčnih mest v reki Temenici.

| Stream reach | Location | Stream order* | Distance from source (km) | Altitude (m) | Mean width (m) | Mean depth (cm) | Maximum temperature (C) |
|--------------|----------------------------|---------------|---------------------------|--------------|----------------|-----------------|-------------------------|
| 1 | Pusti Javor Stranje pri | 2 | 2 | 360 | 1,2 | 6 | 14,2 |
| 2 | Velikem Gabru | 3 | 10 | 300 | 3,1 | 31 | 14,1 |
| 3 | Štefan | 3 | 18 | 280 | 7,3 | 23 | 16,9 |
| 4 | Gorenje Ponikve | 3 | 23 | 270 | 5,9 | 22 | 18,2 |

* After Strahler (1952)

All samples were fixed in 4 % formaldehyde in the field. In the laboratory, organisms were sorted, enumerated, identified under a stereomicroscope and stored in 70 % ethyl alcohol. Macroinvertebrates were determined at least to the family level (except groups Hydrachnida and Collembola) using the keys of BAUERNFEIND & HUMPEŠCH (2001), BRINKHURST (1971), ELLIOT (1977), GERKEN & STERNBERG (1999), GLOER (2002), KARAMAN & PINKSTER (1977), NESEMANN (1997), REYNOLDS (1987), SCHMEDTJE & KOHMANN (1992), TACHET (2000), TRONTELJ & SKET (2000), URBANIČ et al. (2003), WARINGER & GRAF (1997), ŽIŠKO (2000).

Saprobic index was calculated using saprobic and indicative taxa values after WEGEL (1983), MOOG (1995) and URBANIČ (2004) (Trichoptera). Number of taxa per sampling unit (S) and diversity indices (Shannon-Wiener index $H' = -\sum (p_i \cdot \ln p_i)$, Evenness index $E = H'/\ln S$ and Simpson's index $D = 1 - \sum$

p_i^2) were calculated using PC-ORD (McCune & Mefford 1999). One way ANOVAs were performed (using statistical package SPSS) to determine whether the differences in diversity among sampling sites were significant. Multivariate methods used in this study included detrended correspondence analysis (DCA) and canonical correspondence analysis (CCA). Both were performed using statistical package CANOCO. Data on taxa abundances were log transformed. CCA analysis was performed with reduced data matrix which included only sufficiently common taxa (occurrence in at least 2 samples or at least 4 individuals present) and 5 statistically significant variables (forward selection).

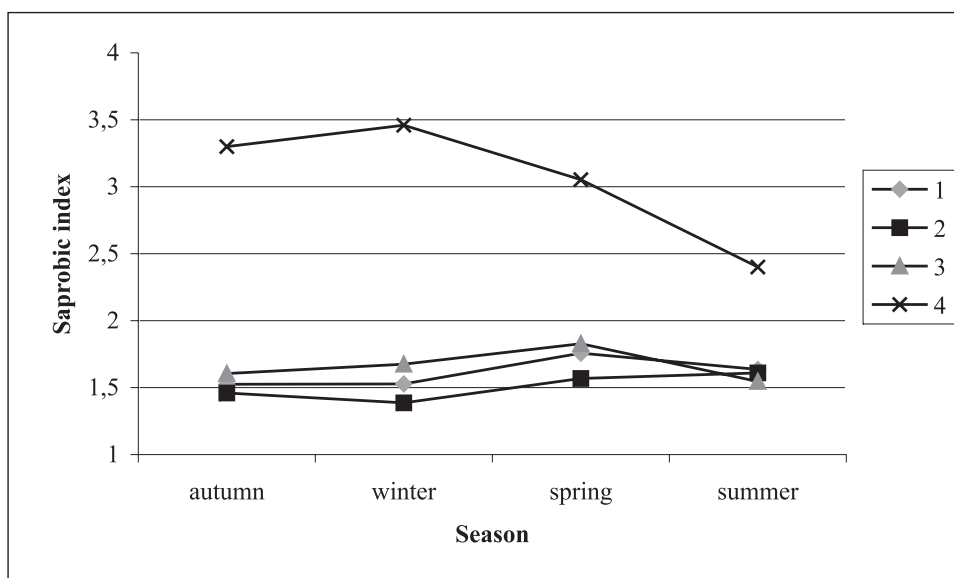


Figure 1: Values of saprobic index at different seasons at four sampling sites of the Temenica river.
Slika 1: Vrednosti saprobnega indeksa v različnih letnih časih na štirih vzorčnih mestih v reki Temenici.

Results

A total of 76550 individuals, representing 120 taxa were collected (Table 2). Saprobic index (Fig. 1) indicates strong organic pollution at sampling site 4 where Tubificidae and Chironomidae were the most abundant. There is an apparent decrease in the value in summertime when Chironomidae (which have a lower saprobic value) outnumbered Tubificidae. Values of the saprobic index at the other three sites were very similar in all seasons and indicate low level of pollution.

Box-plot presentations of taxa number (S) and values of Shannon-Wiener index (H'), Evenness index (E) and Simpson's index (D) are shown in Figure 2. There is a significant decrease in all the values at sampling site 4, which confirms the results of the saprobic index. The number of taxa is also lower at site 1, but diversity indices at this site are equal or even higher (E) than at sites 2 and 3. Using one-way ANOVA we confirmed statistically significant differences in values S, H' and E among sampling sites.

DCA ordination of 16 samples is shown in Figure 3. The first axis explains 40,5 % of the total variance and axis 2 further explains 12,5 %. Samples from each sampling site are clearly separated by the first axis which represents the upstream-downstream gradient and presumably also the pollution gradient. There is a considerable gap between the samples from site 1 and the other samples which indicates significant differences in community structure between the headwaters and the rest of the stream. Axis 2 is apparently related to the seasonal changes in community structure.

Table 2: The abundance of taxa at the sampling sites of Temenica. Abbreviations are added only for taxa used in the CCA

| Sampling site | 1 | 2 | 3 | 4 | Sampling site | 1 | 2 | 3 | 4 |
|------------------------------------|--------------|------|-----|-------|---------------------------------|--------------|-----|-----|-----|
| Taxa | Abbreviation | | | | Taxa | Abbreviation | | | |
| <i>Polycelis felina</i> | 30 | 0 | 0 | 0 | Collembola | 3 | 0 | 0 | 0 |
| <i>Dugesia</i> sp. | 0 | 37 | 8 | 178 | <i>Ephemera danica</i> | 356 | 6 | 2 | 0 |
| <i>Prostoma</i> sp. | 0 | 4 | 0 | 0 | <i>Ephemera major</i> | 0 | 14 | 0 | 0 |
| Mermithidae | 0 | 23 | 3 | 154 | <i>Ephemereilla ignita</i> | 2 | 376 | 153 | 17 |
| <i>Holandriana holandri</i> | 3 | 2232 | 381 | 0 | <i>Caenis macrura</i> | 0 | 0 | 0 | 1 |
| <i>Esperia esperi</i> | 0 | 8 | 367 | 4 | <i>Ecdyonurus</i> sp. | 0 | 4 | 1 | 0 |
| <i>Esperia daudebaritii</i> | 0 | 0 | 83 | 2 | <i>Electrogena</i> sp. | 49 | 5 | 0 | 0 |
| <i>Theodoxus danubialis</i> | 0 | 0 | 174 | 0 | <i>Rhithrogena</i> sp. | 54 | 32 | 1 | 0 |
| <i>Sadleriana fluminensis</i> | 0 | 2 | 0 | 0 | <i>Baetis</i> sp. | 39 | 712 | 244 | 187 |
| <i>Belgrandiella</i> sp. | 0 | 1 | 0 | 0 | <i>Centropilum luteolum</i> | 9 | 0 | 0 | 0 |
| <i>Ancyclus fluvialilis</i> | 0 | 172 | 0 | 3 | <i>Acentrella sinica</i> | 0 | 4 | 0 | 0 |
| <i>Radix bathica</i> | 0 | 1 | 3 | 3 | <i>Habroleptoidea confusa</i> | 35 | 0 | 17 | 1 |
| <i>Physa fontinalis</i> | 0 | 0 | 0 | 12 | <i>Habrophlebia fusca</i> | 67 | 0 | 28 | 1 |
| <i>Pisidium</i> sp. | 1 | 90 | 65 | 86 | <i>Habrophlebia lauta</i> | 2 | 0 | 0 | 0 |
| <i>Helobdella stagnalis</i> | 1 | 0 | 0 | 886 | <i>Paraleptophlebia</i> | | | | |
| <i>Alboglossiphonia hialina</i> | 0 | 0 | 1 | 0 | <i>submarginata</i> | 0 | 0 | 6 | 0 |
| <i>Glossiphonia concolor</i> | 0 | 0 | 0 | 2 | <i>Perloides</i> sp. | 20 | 105 | 55 | 2 |
| <i>Trocheta bykowskii</i> | 0 | 2 | 0 | 0 | <i>Isoperla</i> sp. | 1 | 1 | 0 | 0 |
| <i>Dina</i> sp. | 0 | 0 | 0 | 9 | <i>Nemoura</i> sp. | 33 | 0 | 0 | 0 |
| <i>Eisenella tetraedra</i> | 2 | 41 | 21 | 7 | <i>Protonemura</i> sp. | 16 | 0 | 0 | 0 |
| Lumbriculidae | 0 | 7 | 1 | 0 | <i>Leuctra</i> sp. | 9 | 0 | 3 | 0 |
| <i>Synclritus heringianus</i> | 217 | 699 | 249 | 2219 | <i>Brachyptera</i> sp. | 20 | 4 | 3 | 0 |
| Tubificidae | 4 | 143 | 55 | 25155 | <i>Calopteryx virgo</i> | 0 | 4 | 1 | 0 |
| Naididae | 2 | 32 | 16 | 44 | <i>Calopteryx splendens</i> | 3 | 10 | 0 | 0 |
| <i>Sylaria lacustris</i> | 0 | 0 | 123 | 2 | <i>Platycnemis pennipes</i> | 0 | 0 | 25 | 2 |
| Hydrachnida | 1 | 0 | 0 | 0 | <i>Lestes viridis</i> | 0 | 0 | 0 | 1 |
| <i>Gammareus fossarum</i> | 296 | 863 | 256 | 6 | <i>Lestidae</i> (juv.) | 0 | 0 | 2 | 0 |
| <i>Synurella ambulans</i> | 0 | 0 | 3 | 4 | <i>Gomphus vulgatissimus</i> | 0 | 22 | 14 | 0 |
| <i>Aesellus aquaticus</i> | 1 | 0 | 1 | 47 | <i>Onychogomphus forcipatus</i> | 0 | 11 | 48 | 0 |
| <i>Austropotamobius torrentium</i> | 0 | 0 | 4 | 0 | <i>Onychogomphus uncatatus</i> | 0 | 0 | 17 | 0 |
| | | | | | <i>Cordulegaster boltoni</i> | 10 | 0 | 0 | 0 |
| | | | | | <i>complex</i> | | | | |

| Sampling site | 1 | 2 | 3 | 4 | Sampling site | 1 | 2 | 85 | 0 |
|------------------------------------|--------------|--------------|--------------|--------------|--------------------------------|--------------|-----------|-----------|-----------|
| Taxa | Abbreviation | | | | Taxa | Abbreviation | | | |
| <i>Aphelocheirus aestivalis</i> | 0 | 20 | 85 | 0 | <i>Lepidostoma hirtum</i> | 0 | 1 | 0 | 0 |
| <i>Stalis lutaria</i> | 0 | 0 | 0 | 2 | <i>Potamophylax sp.</i> | 0 | 0 | 5 | 0 |
| <i>Elmis sp.</i> | 14 | 398 | 1361 | 1 | <i>Odontocerum albicorne</i> | 1 | 0 | 0 | 0 |
| <i>Esolus sp.</i> | 61 | 4167 | 1379 | 18 | <i>Wormaldia subnigra</i> | 0 | 0 | 19 | 0 |
| <i>Limnius sp.</i> | 5 | 643 | 131 | 0 | <i>Wormaldia occipitalis</i> | 1 | 0 | 0 | 0 |
| <i>Outimnius sp.</i> | 0 | 346 | 2474 | 33 | <i>Plectrocnemia sp.</i> | 3 | 0 | 0 | 0 |
| <i>Riolus sp.</i> | 7 | 65 | 377 | 1 | <i>Psychomyia klapaleki</i> | 0 | 5 | 0 | 0 |
| <i>Stenelmis sp.</i> | 0 | 0 | 2 | 0 | Psychomyiidae | 3 | 0 | 0 | 0 |
| <i>Hydraena sp.</i> | 61 | 90 | 145 | 5 | <i>Tinodes rostocki</i> | 7 | 0 | 0 | 0 |
| <i>Halipilus sp.</i> | 0 | 0 | 1 | 0 | <i>Lype reducta</i> | 1 | 0 | 0 | 0 |
| Dytiscidae | 0 | 0 | 1 | 0 | <i>Ryacophila tristis</i> | 2 | 1 | 0 | 0 |
| Scirtidae | 276 | 0 | 0 | 0 | <i>Ryacophila sp. (s.str.)</i> | 2 | 3 | 2 | 0 |
| <i>Eubria sp.</i> | 0 | 1 | 0 | 0 | <i>Notidobia ciliaris</i> | 0 | 0 | 2 | 0 |
| <i>Hydropsyche saxonica</i> | 9 | 0 | 0 | 0 | <i>Hydropitila sp.</i> | 1 | 1 | 26 | 5 |
| <i>Hydropsyche pellucidula</i> | 0 | 71 | 8 | 0 | <i>Atherix marginata</i> | 46 | 147 | 15 | 0 |
| <i>Hydropsyche angustipennis</i> | 0 | 0 | 1 | 13 | <i>Atherix ibis</i> | 0 | 6 | 0 | 0 |
| <i>Hydropsyche siltalai</i> | 0 | 11 | 0 | 0 | Anthomyiidae | 0 | 0 | 39 | 1 |
| <i>Hydropsyche sp. (inv.)</i> | 0 | 59 | 4 | 0 | Chironominae | 22 | 563 | 1073 | 13181 |
| <i>Silo piceus</i> | 0 | 571 | 7 | 0 | Orthocladinae | 184 | 292 | 339 | 3818 |
| <i>Silo pallipes</i> | 0 | 1 | 0 | 0 | Tanytopodinae | 14 | 23 | 169 | 843 |
| <i>Agapetus delicatulus</i> | 0 | 54 | 0 | 0 | Dolichopodidae | 0 | 1 | 4 | 0 |
| <i>Glossosoma bifidum</i> | 0 | 1 | 0 | 0 | Ceratopogonini | 0 | 7 | 4 | 12 |
| <i>Athripsodes cinereus</i> | 0 | 8 | 0 | 0 | Empididae | 7 | 13 | 25 | 38 |
| <i>Athripsodes bilineatus</i> | 0 | 5 | 0 | 0 | Limoniidae | 64 | 37 | 21 | 2 |
| <i>Leptocerus interruptus</i> | 0 | 0 | 24 | 0 | Psychodidae | 0 | 1 | 21 | 6 |
| <i>Atella sp.</i> | 0 | 0 | 2 | 0 | <i>Simulium sp.</i> | 54 | 1568 | 2128 | 138 |
| <i>Setodes sp.</i> | 0 | 0 | 1 | 0 | <i>Prosimulium sp.</i> | 41 | 0 | 0 | 0 |
| <i>Mystacides azurea</i> | 0 | 10 | 0 | 0 | Stratiomyidae | 2 | 2 | 1 | 0 |
| <i>Limnephilus lunatus</i> | 0 | 0 | 1 | 2 | Tabanidae | 0 | 15 | 2 | 0 |
| <i>Chaetopteryx major</i> | 17 | 0 | 0 | 0 | Tipulidae | 0 | 1 | 1 | 1 |
| Total number of individuals | 2185 | 14875 | 12334 | 47156 | Total number of taxa | 55 | 69 | 73 | 46 |

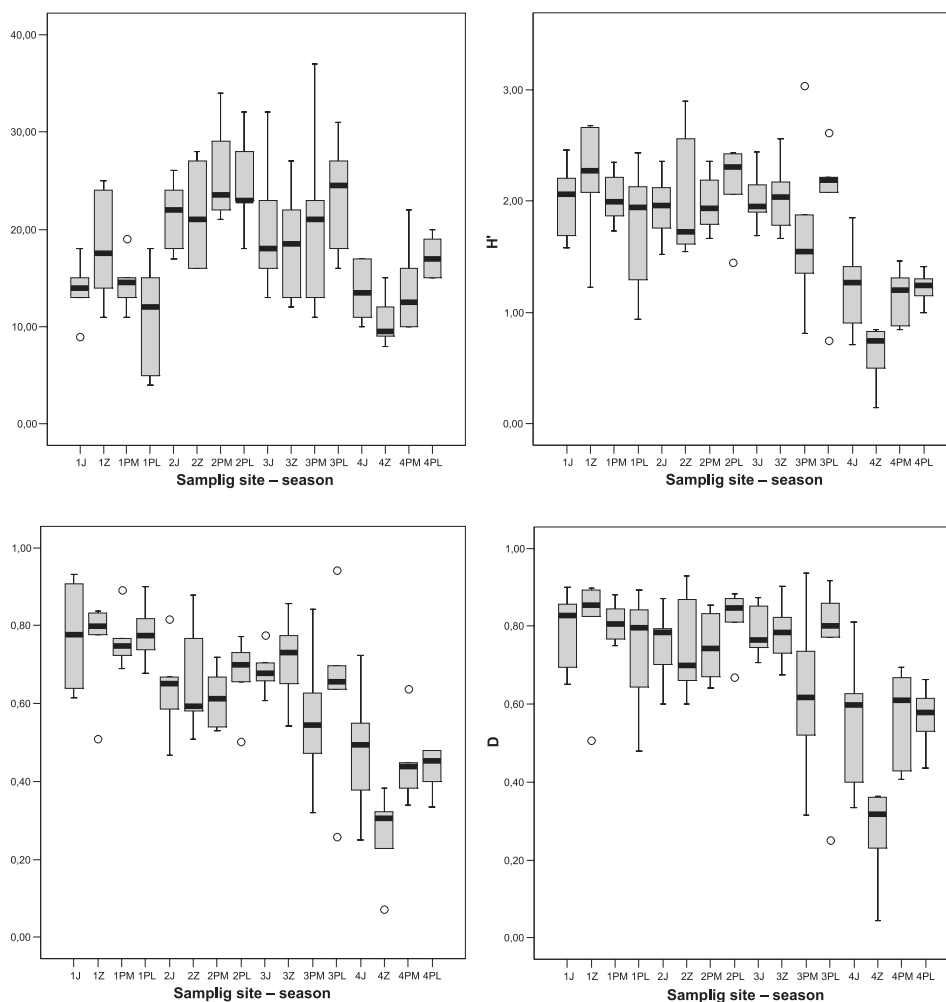


Figure 2: Box-plots of taxa number/sampling unit (S), Shannon-Wiener index (H'), Evenness index (E) and Simpson's index (D) in different seasons at four sampling sites of the Temenica river.

Slika 2: Diagrami kvartilov števila taksonov/vzorčno enoto (S), Shannon-Wienerjevega indeksa (H'), indeksa stalnosti (E) in Simpsonovega indeksa (D) v različnih letnih časih na štirih vzorčnih mestih v reki Temenici.

With 5 selected environmental variables (Fig. 4a) used in the CCA, we explained 64 % of the variance of the taxa-environment relation. Eigenvalues of axes 1 and 2 are 0,38 and 0,28 and display 42 % of the variance. Axis 1 shows high correlation with stream order ($R^2 = -0,95$), and stream width ($R^2 = -0,74$). Taxa which appear on the extreme right side of the biplot (Fig. 4b) therefore prefer headwaters of the stream. Axis 2 is mostly correlated with saprobic index ($R^2 = 0,84$) and may thus present the gradient of organic pollution. Taxa showing affinity (in the upper part of the biplot) to this factor were mostly found at the sampling site 4.

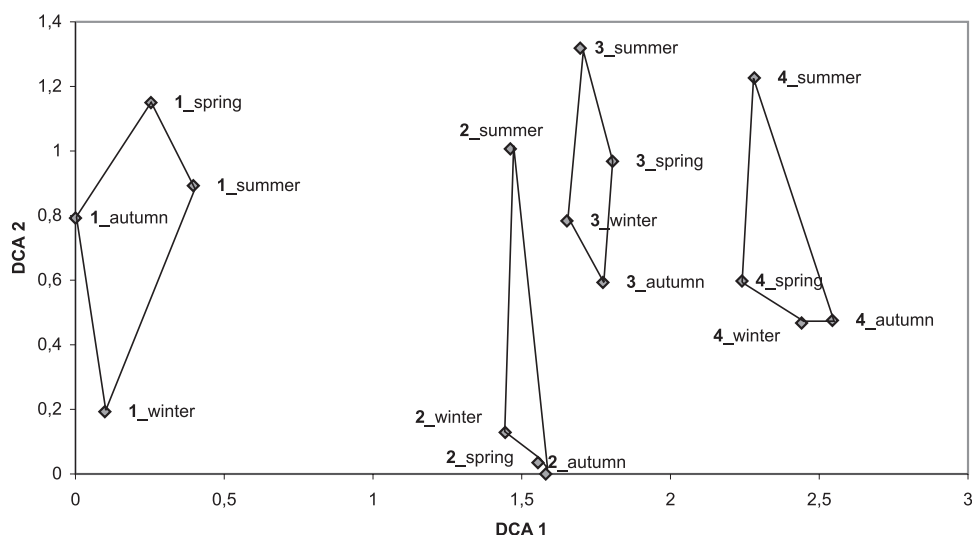


Figure 3: DCA ordination of samples.

Slika 3: DCA ordinacija vzorcev.

Discussion

Macroinvertebrate fauna in the first part of Temenica is relatively diverse with significant differences in community structure among the sampling sites. The fourth site, which lies downstream from Trebnje (population approx. 3000), is especially dissimilar from the rest due to its high saprobic index, low diversity values of indices and the number of taxa. This indicates that the town of Trebnje is a major pollutant of the stream. On the other hand, neither the saprobic index nor the diversity indices showed significant differences among the other three sampling sites.

The values of saprobic index at the fourth sampling site are markedly varying between seasons as a result of changes in abundance of two most abundant groups – Tubificidae (Oligochaeta) and Chironomidae (Diptera). There were also significant seasonal differences in diversity. This indicates the importance of monitoring over a longer period of time.

The first DCA axis can be interpreted both as the upstream-downstream gradient as in the study of ROSSARO & PIETRANGELO (1993) and the pollution gradient, as suggested by Cao et al. (1996). In comparison with the results of saprobic and diversity indices the samples of the sampling site 1 are here clearly separated from the other samples while the differences in community structure between site 4 and the other sites is much less pronounced than in the case of the indices. The reason for this distribution lies in the small number of individuals and different taxonomic structure at site 1, where the morphometric parameters (depth, width etc.) are very different from those at the other sampling sites. The second DCA axis represents seasonal differences among the samples which are very distinct at all four sampling sites.

Group of taxa, found at sampling site 1 (correlated with stream order), is pointing out in the CCA biplot, as well as the taxa, characteristic for site 4 (correlation with saprobic index). This is in agreement with both the indices and the DCA results. Although indices did not show the difference in community structure between the first and the two next sampling sites, they appear to be better at detecting severe pollution (site 4) than multivariate methods. Complementary use of different methods is therefore the most reasonable approach in water quality assessment.

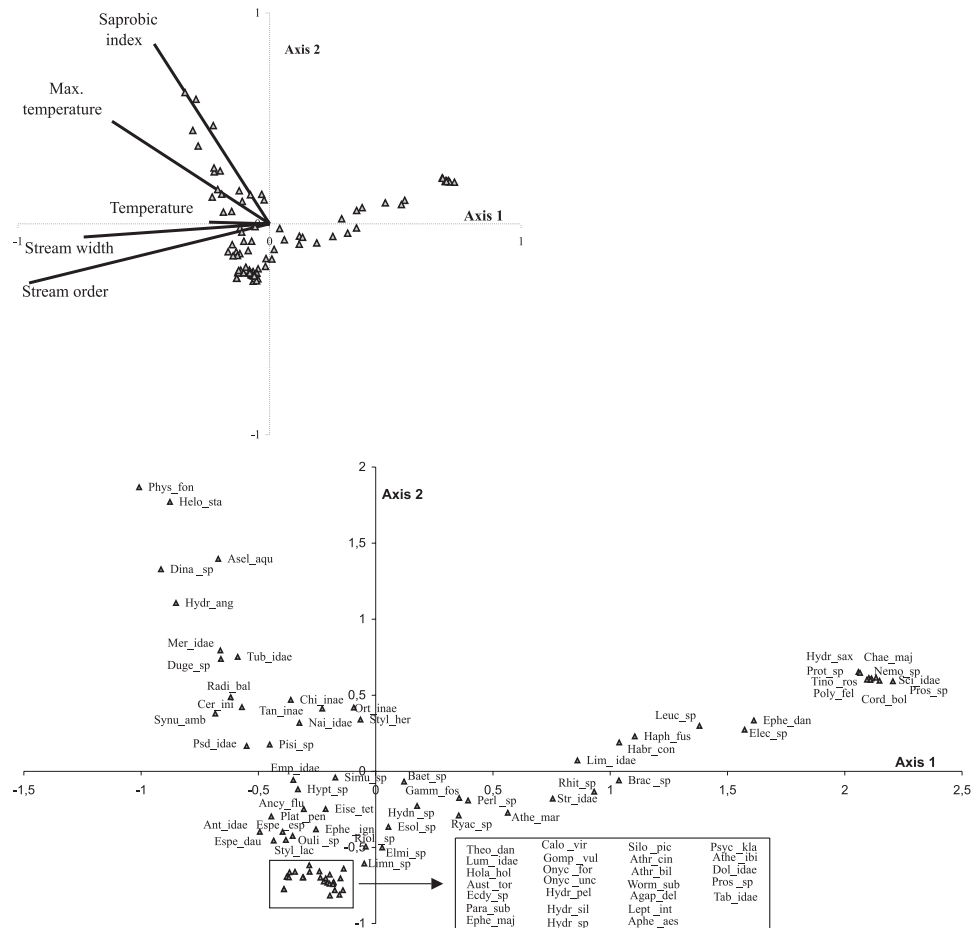


Figure 4: F1 X F2 plane of Canonical correspondence analysis (CCA) between 84 taxa and 5 selected environmental variables. See Table 2 for taxa abbreviations.

Slika 4: F1 x F2 ravnina CCA ordinacijskega diagrama med 84 taksoni in 5 izbranimi okoljkimi spremenljivkami. Glej Tabela 2 za okrajšave taksonov.

Conclusions

1. High values of saprobic index and low values of diversity indices at sampling site 4 indicate that the town Trebnje as the major pollutant of the Temenica river. Differences among other three sampling sites are not detectable with the use of indices.
2. Axis 1 of DCA biplot, representing the upstream-downstream gradient, revealed great differences in macroinvertebrate community structure between the first and the second sampling site, whereas the distinction of the fourth sampling site is much less pronounced. Seasonal changes in community structure are distinct at all sampling sites.
3. Stream order and saprobic index were the most prominent factors affecting the longitudinal distribution of taxa. Taxa significant for sites 1 and 4 were highly correlated with these two factors.

Povzetek

Makroinvertebrati se kot dobri pokazatelji obremenjevanja okolja veliko uporabljajo pri ocenjevanju kakovosti voda. Metode, ki se pri tem uporabljajo pa temeljijo na različnih principih in lahko dajejo različne informacije o vodnem okolju. Zaradi številnih pomanjkljivosti indeksov, ki se običajno uporabljajo za rutinski biomonitoring celinskih voda, se v vrednotenju kakovosti čedalje pogosteje uporabljajo multivariatne metode. S temi lahko zaznamo manjše spremembe v združbi kot z indeksi, dajo pa nam lahko tudi informacije o odzivu taksonov na posamezne okoljske spremenljivke.

V naši raziskavi smo z analizo združbe makroinvertebratov ocenjevali ekološko stanje prvega dela ponikalne reke Temenice, pri čemer smo uporabili različne metode, da bi preverili njihovo uporabnost pri ocenjevanju kakovosti voda. Kvantitativno vzorčenje s Surberjevim vzorčevalnikom smo izvajali vsake tri mesece od oktobra 2003 do julija 2004 na štirih vzorčnih mestih. Vrednosti saprobnega indeksa, diverzitetnih indeksov ter število taksonov na vzorčno enoto kažejo na močno poslabšanje kakovosti reke Temenice na zadnjem vzorčnem mestu v primerjavi s prvimi tremi in s tem na močan negativen vpliv mesta Trebnje na njeno ekološko stanje. Razvrstitev vzorcev po prvi DCA osi potrjuje našo domnevo o naraščanju obremenjevanja reke po toku navzdol, pri čemer pa četrto vzorčno mesto v primerjavi z rezultati indeksov ni tako izstopajoče. Večje razlike v združbi makroinvertebratov so po tej analizi med prvim vzorčnim mestom in ostalimi tremi. Na vseh vzorčnih mestih so izrazite tudi sezonske razlike v strukturi združbe. Najpomembnejši spremenljivki za razporeditev taksonov sta po rezultatih CCA red vodotoka in vrednost saprobnega indeksa, kar izpostavi taksone, ki so bili najbolj zastopani na prvem in zadnjem vzorčnem mestu.

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