EPHEMEROPTERA AND PLECOPTERA LARVAE AS ENVIRONMENTAL INDICATORS IN RUNNING WATERS OF ESTONIA

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Qualitative macrozoobenthos samples from Estonian fast-flowing stream stretches, collected simultaneously with water samples in spring, summer and autumn 1990-1993, were analysed. The ASPT index (ARMITAGE *et al.*, 1983), distance from the stream source, and 10 hydrochemical parameters were estimated for 31 species, 11 families of Ephemeroptera and Plecoptera. Covariance analysis was used for the comparison of different species in relation to environmental parameters. A new index based on the sensitivity of macroinvertebrate families to water quality was established by ranking the mean and pessimum values of the pH, total P, BOD7, and NH_4.

INTRODUCTION

The use of freshwater macroinvertebrates as environmental indicators is widespread (ROSENBERG & RESH, 1993), especially in Europe where several such systems to estimate water quality have been developed (METCALFE, 1989). The possibilities of rapid and cost-effective approaches were discussed by several authors (PLAFKIN *et al.*, 1989; RESH *et al.*, 1995). In most cases, the order Plecoptera (or its components) is considered as the most sensitive to organic pollution and lack of oxygen (WOODIWISS, 1964; VERNEAUX *et al.*, 1982; ARMITAGE *et al.*, 1983; LENAT, 1988; FRIEDRICH, 1990). The order Ephemeroptera is often regarded as the second most sensitive to environmental stress after Plecoptera.

There have been few attempts to use macroinvertebrates for the estimation of water quality in the Estonian watercourses (SEIRE, 1994; TIMM, 1991; 1995b). After REMM (1970), there are 41 species of Ephemeroptera in Estonian waters. 16 species of Plecoptera were recorded in Estonian running waters in 1985-1990 (TIMM, 1995a).

The project «Elaboration of the bioindication methods for the estimation of the state of environment» was realized on Estonian running waters in 1990-1993 by the Institute of Environmental Protection (Estonian Agricultural Institute) using the taxonomical composition of macroinvertebrates. The suitability of some water quality indices was estimated on Estonian streams. The Average Score Per Taxon (ASPT) (ARMITAGE *et al.*, 1983) revealed the best correlations with the main hydrochemical parameters and had the least variability as compared to other indices. Therefore, it was used as a preliminary standard water quality indication tool in Estonian running waters.

STUDY AREA, MATERIAL AND METHODS

Estonia as a part of the East-European platform is characterized by an even surface with the average elevation of 50 metres. Among the over 7000 watercourses, only 9 reach 100 km or more (ARUKAEVU, 1986).

Pollution with total N and NO_3-N (>2.8 mg/l and 1.2 mg/l, respectively) is widespread in rivers based on limestone bedrock (in North Estonia), caused by the high content of nitrogen in groundwater (STARAST, 1983; JÄRVEKÜLG & VIIK, 1991). The distribution of NO_2-N and NH_4-N pollution has no regional differences. High PO_4 content (>0.03 mg/l) is often found in South Estonian rivers (based on sandstone) as well as downstream of large settlements. Total P content is the key factor causing eutrophication in most of the South Estonian rivers (JÄRVET, 1991). The pH usually fluctuates between 7 and 8.5 (SIMM, 1975). Acid precipitation is often neutralized in the atmosphere and has no significant effects on the quality of soil or surface waters (FREY *et al.*, 1991).

289 qualitative samples of macroinvertebrates as well as water samples from 54 permanent watercourses were collected in spring (May), summer (August), and autumn (October) 1990-1993 (Fig. 1). Several streams suffered under serious agricultural pollution (especially due to organic fertilizers). The stretches with the current rate >= 0.3 m/s (in spring) were preferred. The mean distance to the stream source was 19 ± 2 km (range 1-106 km).

A sample consisted of animals taken from hard substrates, such as stones, branches, aquatic vegetation; and sometimes also from sediment (sand, gravel) collected with a bottom scraper. Macroinvertebrates were fixed *in situ* in 70% ethanol. The sampling and sorting time was limited to 30 min. per sample.

Hydrochemical analyses were made in the laboratory of «ELVI-Aqua» (Tartu); the pH and oxygen contents were

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measured *in situ*. The Average Score Per Taxon (ASPT) index was calculated to estimate water quality on the basis of macroinvertebrates, according to ARMITAGE *et al.* (1983). Covariance analysis with Bonferroni correction was used to relate the presence-absence data of different taxa with environmental parameters (SAS, 1991). The mean values of the parameters were calculated separately for samples with and without certain taxa.

RESULTS

Table 1 presents the mean values of environmental parameters measured at macroinvertebrate localities. NEWMAN (1988) regarded pH, O_2, BOD, and NH_4 as the main parameters used for estimating water quality in the European Community countries. Water quality standards after NEWMAN (1988) and STARAST (1983) are the following: pH - 6.5-8.5; BOD7 - <3 mg O/l; total P - <0.1 mg/l; PO_4 - <0.03 mg/l; NH_4 - <0.4 mg/l; NO_2 - <0.02 mg/l; NO_3 - 1.2 mg/l. All mean values of the parameters except NO_3 were lower than the standards in the watercouses studied. The high NO_3 contents were probably caused by fertilizer-polluted groundwaters. Summer appeared an unfavourable season as evidenced by minimal O_2, maximal BOD7, and also maximal concentrations of most biogenic compounds. At the same time, the ASPT did not reveal seasonal differences, as the number of taxa was minimal in autumn.

In all, larvae of 18 ephemeropteran and 13 plecopteran species were found: *Baetis buceratus* EATON, *B. muticus* (LINNAEUS), *B. niger* (LINNAEUS), *B. rhodani* (PICTET), *B. vernus* CURTIS, *Centroptilum luteolum* (MÜLLER), *Cloëon dipterum* (LINNAEUS), *Caenis horaria* (LINNAEUS), *Brachycercus harrisella* CURTIS, *Siphlonurus alternatus* (SAY), *Heptagenia fuscogrisea* (RETZIUS), *H. sulphurea* (MÜLLER), *Ephemera danica* MÜLLER, *E. vulgata* LINNAEUS, *Ephemerella ignita* (PODA), *Habrophlebia fusca* (CURTIS), *Leptophlebia vespertina* (LINNAEUS), and *Paraleptophlebia submarginata* (STEPHENS); *Taeniopteryx nebulosa* (LINNAEUS), *Amphinemura standfussi* (RIS), *Nemoura cinerea*

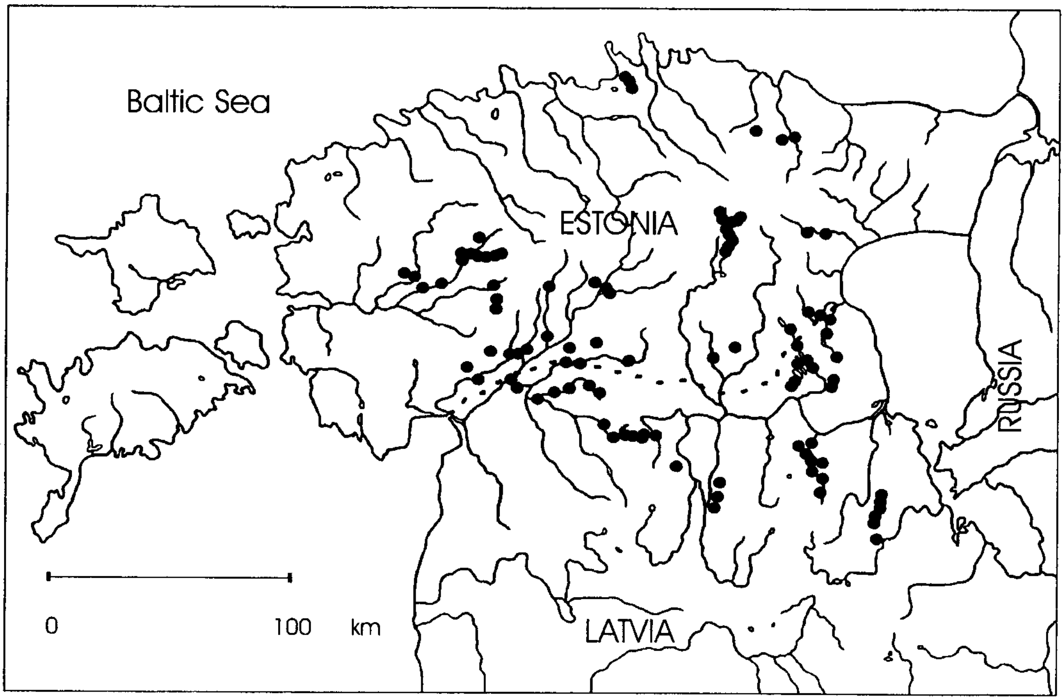

Fig. 1. Study area. The broken line indicates the approximate border between the bedrock types: Ordovician and Silurian limestones (northern), and Devonian sandstones (southern).

(RETZIUS), *N. dubitans* MORTON, *N. flexuosa* AUBERT, *Nemurella pictetii* KLAPÁLEK, *Leuctra digitata* KEMPNY, *Diura bicaudata* (LINNAEUS), *Isogenus nubecula* NEWMAN, *Isoperla grammatica* (PODA), *I. difformis* (KLAPÁLEK), *Perlodes dispar* (RAMBUR), and *P. microcephalus* (PICTET).

Larvae of many ephemeropteran species occurred only in spring or also in summer. The presence of two *Ephemera* species throughout the period could probably be explained by their long life-cycle (more than 1 year). *Ephemerella ignita* was found in all seasons but most abundantly in summer. A very common species *Heptagenia sulphurea* surprisingly occurred in all the seasons studied. Larvae of the majority of plecopteran species occurred in spring. *Leuctra digitata* was present in summer; *Taeniopteryx nebulosa* and *Diura bicaudata* were found only in autumn.

The following significant (≤0.05) preferences were detected: *Baetis muticus* preferred high O_2, high alkalinity, and high PO_4; *B. niger* - high NH_4; and *B. rhodani* - high pH. The ASPT index was significantly higher in the samples where *Heptagenia sulphurea, Ephemera danica,* or *Ephemerella ignita* were found. Both genus *Ephemera* and the whole order were found in the samples with high ASPT index. Scarcity of data (less than 10 measurements) probably diminished the efficiency of the analysis in the case of *B. buceratus, B. vernus, B. harrisella, H. fuscogrisea,* and *H. fusca. T. nebulosa* preferred the high ASPT and long distance from the river source, *I. grammatica* - high ASPT and oxygen content; *I. nubecula* - long distance from the river source, *L. digitata* - high pH. The family Isoperlidae was found in samples with high ASPT and relatively long distance from the source. The whole order Plecoptera preferred the high ASPT and high pH, respectively. Nevertheless, scarcity of data (less than 10 measurements) probably diminished the efficiency of analysis in the case of *D. bicaudata, I. nubecula, I. difformis, Perlodes* spp., and *N. flexuosa.*

A new index based on the sensitivity of macroinvertebrate families to pH, BOD7, total P, and

Table 1. Environmental parameters in watercourses. BOD7 - mgO/l; other measures except pH, ASPT, and the number of taxa - mg/l.

Parameter	Spring	Summer	Autumn	Mean	Min.	Max.
pH	7.6	7.8	7.5	7.6	4.6	9.1
BOD7	2.3	3.1	1.8	2.4	0.2	8.8
Total P	0.08	0.10	0.07	0.08	0.01	0.90
PO4	0.04	0.08	0.06	0.06	0.01	0.50
NH4	0.25	0.32	0.27	0.28	0	3.3
NO2	0.016	0.017	0.013	0.015	0	0.240
NO3	1.8	1.3	2.1	1.7	0	10.0
Alkalinity	5.68	5.85	5.77	5.77	1.77	7.54
O2	10.0	7.9	8.7	8.9	4.2	14.0
ASPT	5.4	5.5	5.4	5.4	1.0	8.4
Mean number of taxa	10.9	10.6	7.7	9.7	1	23

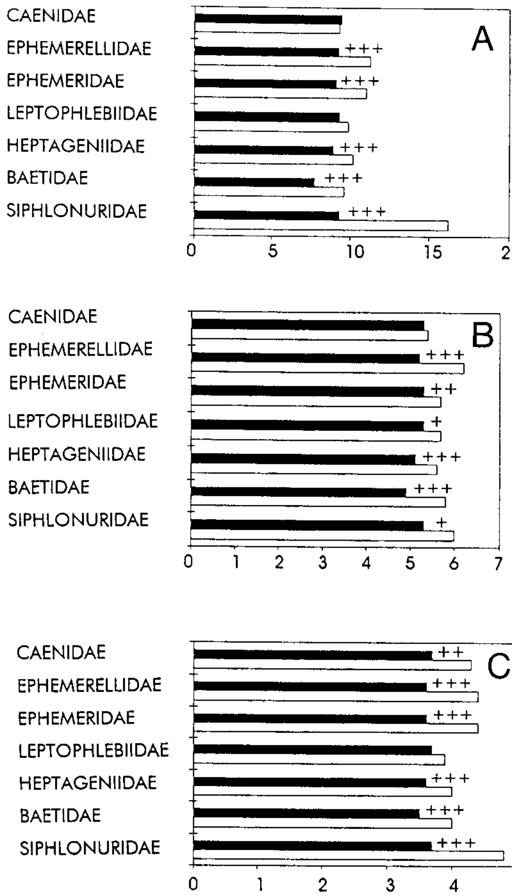

Fig. 2. Ephemeroptera. A: Mean number of taxa (Ntaxa); B: Average Score Per Taxon (ASPT) (ARMITAGE *et al.* 1983), C: the original biotic index (EI). Black bars: the mean value of parameter when the target family is absent; white bars: standardized mean value of the parameter when the target family is present. Significance levels: +++ - 0.01, ++ - 0.05, + - 0.1.

Table 2. Mean tolerance values of the ephemeropteran and plecopteran families on the 10-point scale (the highest number indicating the lowest tolerance). ASPT - ARMITAGE *et al.* (1983), EI - original results, IBQG - VERNEAUX *et al.* (1982), HBI - HILSENHOFF (1988). S - theoretical value, calculated from the mean saprobity indices of other references (ANDERSEN *et al.*, 1983; FRIEDRICH, 1990; JANEVA, 1979; MAKRUSIN, 1974; MOLLER PILLOT, 1971; RUSSEV, 1979; RUSSEV *et al.*, 1976; SCHMEDTJE & KOHMANN, 1987; STJERNA-POOTH, 1981; WEGL, 1983; ZELINKa, 1978).

Family	ASPT	EI	IBQG	10-HBI	S
Siphlonuridae	10	8	–	3	–
Heptageniidae	10	4	5	6	7.1
Leptophlebiidae	10	7	7	8	7.8
Ephemerellidae	10	7	4	9	6.8
Ephemeridae	10	6	6	6	6.9
Caenidae	7	8	3	7	6.8
Baetidae	4	3	3	4	7.2
Taeniopterygidae	10	8	9	8	7
Leuctridae	10	7	7	10	7.8
Perlodidae	10	7	8	8	8.6
Nemouridae	7	4	5	8	8.5

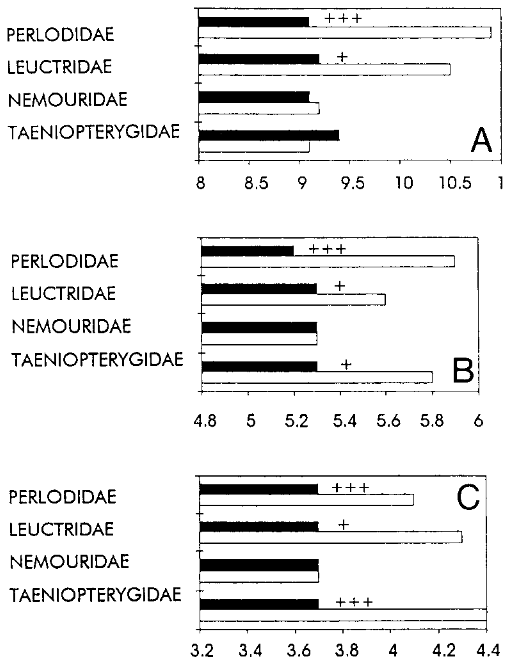

Fig. 3. Plecoptera. A: Mean number of taxa (Ntaxa); B: Average Score Per Taxon (ASPT) (ARMITAGE *et al.* 1983), C: the original biotic index (EI). Black bars: the mean value of parameter when the target family is absent; white bars: standardized mean value of the parameter when the target family is present. Significance levels: +++ - 0.01, ++ - 0.05, + - 0.1.

NH_4 values was composed as follows. The mean and pessimum (the lowest) values of pH were ranked in the descending, and these of the other parameters (the maximum values considered as pessimal) in ascending order. Because of the low occurrence of many single species as well as difficulties in the identification of young larvae, the family level was used to reduce information loss. The sensitivity of a family was calculated as the mean rank numbers of the four parameters. Similarly to ASPT, the sensitivity scale was divided into 10 parts so that the most sensitive taxa were marked with 10 points, and the most tolerant ones with 1 point. The new index (EI) was calculated for all single samples and compared its sensitivity to ASPT and to the number of macroinvertebrate taxa in a sample (ANOVA, Figs 2, 3). For standardization, ASPT and EI were calculated without the target family (if present) in every case; 1 was subtracted from the number of taxa when the target family was present. As a rule, the number of taxa, ASPT, and also EI appeared significantly higher in presence of every ephemeropteran family than in samples without them (except quite rare, partially standing-water families of Caenidae and Leptophlebiidae). The number of taxa, ASPT, and also the EI index always appeared significantly higher in the presence of Perlodidae and Leuctridae than in their absence. In the case of Taeniopterygidae, the low number of taxa in the presence-data was probably caused by its occurrence only in autumn. Nemouridae never revealed analogous significant differences.

Thus, the sensitivity of some Ephemeroptera and Plecoptera larvae to some environmental parameters was confirmed in Estonian running waters. Many taxa and the whole orders preferred the most versatile biotopes (as estimated by ASPT and EI indices). The occurrence of the larvae mainly in spring, and the low density of many sensitive taxa is limiting the use of Ephemeroptera and Plecoptera for the estimation of water quality.

DISCUSSION

Table 2 demonstrates the tolerance values of certain families after different authors, converted to 10-point system with ascending sensitivity. The values of the saprobic indices from plenty references were transformed to the corresponding ASPT values, using the nomogram of SLADEČEK & TUČEK (1975), and the original measurements of BOD. In the last case, only the species also found in the Estonian watercouses were used. The approximate relation between ASPT and saprobic index (s) was derived: ASPT ≈ -3.0 s + 13.0.

The differences are probably caused by the different initial parameters or the regional variation. For example, if ASPT was originally related to the «wide range of physical and chemical features», then EI was dependent on only 4 hydrochemical measures. HBI and IBQG were based on organic and nutrient pollution in general; IBQG also required numerous combinations of substrate and velocity conditions in each site to be assessed. The uniformity of the values derived from BOD-based saprobic indices is probably caused by the low number of saprobity classes. Most of the plecopteran species were regarded to be O- or β-mesosaprobic indicators while the ephemeropteran species were considered mainly β-mesosaprobic.

The genera *Baetis* and *Caenis* were regarded as the most tolerant to organic pollution (BARGOS *et al.*, 1990; CORTES *et al.*, 1986), especially in fast flow. Heptageniidae and Ephemeridae, on the contrary, were considered as intolerant groups (MOLLER PILLOT, 1971). Nevertheless, as compared to some other invertebrate groups, *Baetis rhodani* demonstrated the highest sensitivity to water ammonium content (WILLIAMS *et al.*, 1986). On the other hand, *Cloeon dipterum* in the River Rhine appeared as the species most tolerant to 15 selected toxicants, even as compared to Chironomidae and Tubificidae (SLOOFF, 1983). ENGBLOM & LINGDELL (1987) presented pH tolerance limits for some ephemeropteran species in Swedish freshwaters. The highest mean value were revealed by *Baetis muticus* (7.3) and *Ephemera danica* (7.2), and the lowest - *Leptophlebia vespertina* (6.3). Nevertheless, *L. vespertina* and *Cloeon dipterum* were found with pH also as high as 10. *Ephemerella ignita* and *Baetis muticus* disappeared with pH ≤6.6, caused by the lack of food; *B. rhodani* was able to survive such low pH in the condition of high ionic loadings (WILLOUGHBY, 1988; WILLOUGHBY & MAPPIN, 1988).

Water pH appeared to be a good indicator in the estimation of biotopes of Plecoptera larvae. ENGBLOM & LINGDELL (1987) presented an extensive list of living macroinvertebrate species with pH tolerance. *Amphinemura standfussi* (7.1), *Leuctra digitata* (7.0), *Taeniopteryx nebulosa* and *Isogenus nubecula* (both 6.9) revealed the highest mean values, while *Nemoura cinerea* (6.3) and *Nemurella pictetii* (6.4) represented the minimal values. *N. pictetii* preferred low pH also in British rivers (TOWNSEND *et al.*, 1983). RUPPRECHT (1991) suggested its pH tolerance limits as wide as 3-8.5. Taeniopterygidae and Perlodidae avoided acid waters (RUTT *et al.*, 1990).

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