# The use of Ephemeroptera to assess aquatic biodiversity in the rhithral part of the Luxembourgish rivers

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### **Abstract**

In a multi-field survey conducted during the period 1994-1998, 39 species of Ephemeroptera were recorded in the rhithral part of the Luxembourgish rivers. They belong to 22 genera and represent 8 families out of the 15 encontered in Europe. The relationship of these macroinvertebrates between their distribution and the abiotic/biotic factors were elucidated with the multivariate Correspondance Analysis (partial CCA) technique. The results of the partial CCA ordination using environmental variables obtained by forwarded selection show that 9.8 % of the variance in species occurrences are accounted by the first two axes and 73.1 % of the variation could be explained by the environmental variables. The four main factors having an important significance for larvae were the conductivity, the slope, the organic pollution and the stream order. Ephemeroptera constitute a very interesting group for studying running water and can therefore be considered as a useful group for assessing aquatic biodiversity in rivers.

**Keywords**: mayflies, indicator species, ordination, water quality, Luxembourg.

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#### Introduction

The Ephemeroptera display ecological and behavioural specializations which enable them to colonize a wide range of lotic and lentic waters. In addition, Ephemeroptera are abundant and represent an important component of the biotic diversity in streams of Luxembourg. In a precedent analysis, Ephemeroptera proved to be the third most diverse group, just after Diptera and Trichoptera and followed by Coleoptera, Plecoptera and other groups of macroinvertebrates (Dohet, 2000).

The aim of this survey is to characterize the Ephemeroptera fauna of the study area and to detect the main spatial pattern of distribution across environmental gradients.

#### Material and Methods

Study area

Luxembourg is divided into two major defined regions: the homogeneous schistous hercynian massif in the North (Oesling) with a mean altitude approaching 500 m and the Triassic and Jurassic layers on a Devonian base in the South (Gutland). The total area represents 2586 km² (Fig. 1) The climate in Luxembourg can be considered as temperate. The Oesling is a wooded area with low anthropogenic disturbances. On the contrary, the Gutland is more influenced by human activities. 149 sites were chosen over the whole country in order to cover the main types of rivers as well as different degrees of human impact.

Species data

39 species belonging to 8 families of Ephemeroptera were recorded in the rhithral part of the Luxembourgish rivers (Dolisy, 2000). The most diverse families are Baetidae and Heptageniidae each containing 13 and 11 species of all species, respectively. Within the Baetidae the most diverse genera is *Baetis* which contains 8 species. In the other families specific diversity is more equally distributed and contains between 1 and 4 species. Species names and codes used in CCA are listed in Table 1.

## Environmental data

149 collecting sites in the rhithral part of the streams were chosen over the whole country in order to cover the main types of rivers (Fig. 1). Aquatic biotopes were identified and a semi-quantitative method was employed to sample each of them. Hand nets were used to collect larval

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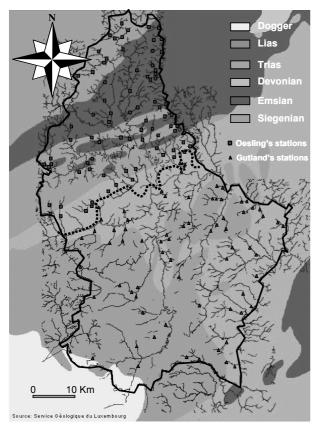


Fig. 1 - Map depicting the hydrological network of Luxembourg and showing the stations chosen. The dotted line separates the Oesling area (square symbol) from the Gutland area (triangle symbol).

stages twice a year (between spring 1994 and autumn 1998). Water samples were collected and analysed following standard procedures in the laboratory of the Environment Administration of Luxembourg, respectively the laboratory of CRP-GL, for the following variables: chlorides, sulphates, total phosphorus, ortho-phosphates, nitrates, nitrites, ammonium, sodium, potassium, total hardness and BOD5. To assess organic pollution, a multimetric index "IPO" (Leclercq and Maquet, 1987) based on four parameters (BOD5, NH4, NO2 and PO4) was calculated. The values of the IPO are based on four parameters: NH4+, NO2-, PO4--- and BOD5. The scale ranges from 1 (low organic pollution) to 5 (high organic pollution). Different physical and biological variables (stream width, stream depth, stream length, substrate number, bottom substrate type, aquatic vegetation) of the collecting sites were noted in the field and geographic information on the location (latitude, longitude, L\*l, distance from the spring source, stream order, altitude, slope) was derived from 1/20.000 topographical maps. For the CCA analysis, the substrate types were summarized in three categories: stones

(bedrock, cobbles), sediment (coarse and fine gravels, sand, silt) and vegetation (litter, roots, submerged and emergent aquatic vegetation, moose). The range values (min., max., 25-75% quartiles, median) of 22 environmental variables for each site were summarized in Table 2.

Table 1 - List of species code and full species name used in CCA.

useu III CCA	
ALA MUT	Alainites muticus (LINNE, 1758)
BAE ALP	Baetis alpinus (PICTET, 1843)
BAE FUS	Baetis fuscatus (LINNE, 1761)
BAE LUT	Baetis lutheri MÜLLER-LIEBENAU, 1967
BAE MEL	Baetis melanonyx (PICTET, 1843)
BAE RHO	Baetis rhodani (PICTET, 1843)
BAE SCA	Baetis scambus EATON, 1870
BAE SP	Baetis sp. LEACH, 1815
BAE VAR	Baetis vardarensis IKONOMOV, 1962
BAE VER	Baetis vernus Curtis, 1834
CAE BES	Caenis beskidensis SOWA, 1973
CAE HOR	Caenis horaria (LINNÉ, 1758)
CEN LUT	Centroptilum luteolum (MÜLLER, 1776)
CLO DIP	Cloeon gr-dipterum (LINNÉ, 1761)
ECD DIS	Ecdyonurus dispar (CURTIS, 1834)
ECD SP	Ecdyonurus sp. Eaton, 1865
ECD SUB	Ecdyonurus submontanus LANDA, 1869
ECD TOR	Ecdyonurus torrentis KIMMINS, 1942
ECD VEN	Ecdyonurus venosus (FABRICIUS, 1775)
ELE LAT	Electrogena lateralis (CURTIS, 1834)
ELE UJH	Electrogena ujhelyii (SOWA, 1981)
ELE SP	Electrogena sp. ZURWERRA & TOMKA, 1985
EPE SYL	Epeorus sylvicolus (PICTET, 1865)
EPH DAN	Ephemera danica Müller, 1764
EPH MUC	Ephemerella mucronata (BENGTSSON, 1909)
HAB CON	Habroleptoides confusa SARTORI & JACOB, 1986
HAB FUS	Habrophlebia fusca (CURTIS, 1834)
HAB LAU	Habrophlebia lauta EATON, 1884
HEP SP	Heptageniidae
LEP MAR	Leptophlebia marginata (LINNÉ, 1767)
MET BAL	Metreletus balcanicus (ULMER, 1920)
NIG NIG	Nigrobaetis niger (LINNÉ, 1761)
PAR SUB	Paraleptophlebia submarginata (STEPHENS, 1835)
PRO BIF	Procloeon bifidum (BENGTSSON, 1912)
RHI HER	Rhithrogena hercynia LANDA, 1969
RHI HYB-GR	Rhithrogena hybrida-Gr
RHI PIC	Rhithrogena picteti (SOWA, 1971)
RHI PUY	Rhithrogena puytoraci SOWA & DEGRANGE, 1987
RHI SEM	Rhithrogena semicolorata (CURTIS, 1834)
RHI SEM-GR	Rhithrogena semicolorata-Gr
SER IGN	Serratella ignita (PODA, 1761)
SIP AES	Siphlonurus aestivalis (EATON, 1903)
SIP LAC	Siphlonurus lacustris (EATON, 1870)
TOR MAJ	Torleya major (KLAPALEK, 1905)

# Multivariate analysis Ordination

Environmental variables that may affect the distribution of mayflies were investigated by Canonical Correspondance Analysis (CCA) using the CANOCO program of ter Braak and Smilauer (1998). A log-transformation was made to reduce the effect of a highly abundant species (Jackson 1993, Cao *et al.*, 1997). The mayflies were sampled in two different seasons (spring and autumn). This seasonal variation being not the prime objective of the present paper, a partial canonical correspondance analysis (partial CCA) in which the two class variables representing

seasons were treated as covariables was used (ter Braak and Verdonschot, 1995). Partial CCA was conducted using the data set containing 59.645 specimens belonging to 45 taxa in 296 samples. Environmental variables were recorded for the same samples. Species and samples were ordinated along axes produced by linear combinations ofenvironmental variables Environmental variables are shown on plots as arrows: the longer the arrow and the closer it is to an axis, the more important it is in defining that axis. Significance of the relation between the species and the environmental variables were tested using a Monte Carlo Random Re-sampling Test using 199 random permutations. Results of

CCA were plotted for the first two canonical axes using the computer program Canodraw (ter Braak, Smilauer, 1998)

#### Results

The results of the CCA ordination show that 9.8 % of the variance in species occurrences are accounted by the first two axes and 73.1 % of the variation could be explained by the environmental variables (Table 3).

Only the six environmental variables most strongly correlated to the species data are shown in the ordination diagram (Fig. 2).

Table 2 - Different range values (min-max, 75%-25% quartiles, median) of environmental variables from autumn 94 to spring 98. Abbreviations are: IPO: index of organic pollution, Alt.: altitude, SD: source distance, TH: total hardness (French degree), THCa: carbonate hardness (French degree).

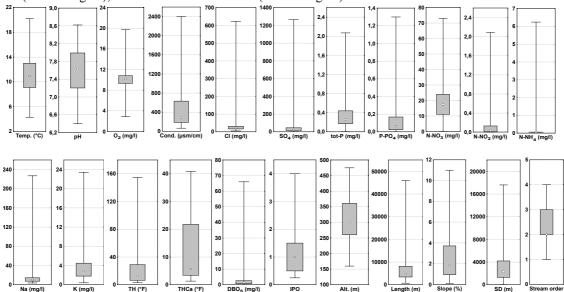


Table 3 - Results of canonical correspondance analysis (CCA) using environmental variables obtained by forward selection procedure for four axes of the CCA and the significance of the Monte Carlo test for the first axis. Weighed intraset correlations between axes 1 to 4 and the 6 environmental variables forwarded.

<u>Axes</u>	1	2	3	4	Total inertia	
Eigenvalues:	.189	.104	.054	.028	3.054	
Species-environment correlations:	.715	.706	.552	.368		
Cumulative percentage variance						
of species data:	6.3	9.8	11.6	12.6		
of species-environment relation:	47.1	73.1	86.4	93.4		
Sum of all unconstrained eigenvalues = 2.979 Sum of all canonical eigenvalues = 0.401 Monte Carlo permutation test F=18.525 (p<0.005)						
Conductivity	0.537	0.306	-0.170	0.005		
Slope	-0.368	0.490	0.177	0.010		
Organic Pollution Index (IPO)	0.489	-0.067	0.281	-0.094		
Stream Order (SO)	-0.013	-0.542	0.093	-0.123		
Carbonate hardness (THCA)	0.052	0.306	-0.215	-0.023		
T°	0.053	-0.226	0.144	0.296		

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They are in decreasing order of importance: conductivity, slope, organic pollution (IPO), stream order, carbonate hardness and temperature. The first axis is positively correlated with the conductivity (0.537), organic pollution (0.489), carbonate hardness (0.052) and negatively correlated with slope (-0.368). The second axis indicates a negative correlation mainly with stream order (-0.542) and temperature (-0.226).

The stations are clearly distributed along the gradient of mineralisation. The high values of water mineralisation indicated by the conductivity and total carbonate hardness (THCa) attest of the different geological nature of the two regions prospected: Oesling (square symbol) and Gutland (triangle symbol).

Ephemeroptera are well dispersed over the whole CCA ordination diagram, but it can be noted that most of them are positioned in the

lower left quarter. Those species occured in streams characterized by low mineralisation and high-order stream. It can be noted Ephemeroptera are also well distributed along the stream order gradient and therefore constitute a very interesting group for studying longitudinal zonation. Species of fast flowing water and low anthropogenic disturbance occurred in the upper left quarter. Species of high mineralisation and low-order stream occurred in the upper right one. the lower right Species in quarter characterized by moderate anthropogenic disturbance and high-order stream. multimetric index that characterizes organic pollution (IPO) confirms the relatively good water quality in the investigated stations. Most values are ranging between 0.4 and 1.5 on a scale of 1 to 5.

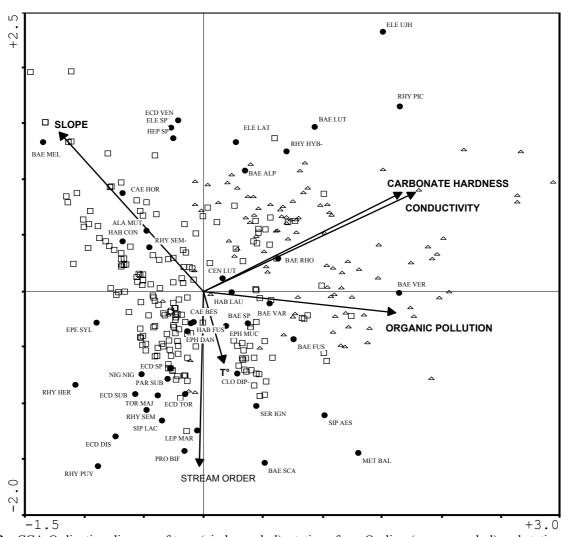


Fig. 2 - CCA Ordination diagram of taxa (circle symbol), stations from Oesling (square symbol) and stations from Gutland (triangle symbol). Eigenvalues and percent variance of species data are listed for the first four axes of CCA. F-ratio statistics (Monte Carlo permutation test) are listed for the first axis and all axes combined.

Considering the organic pollution gradient, Ephemeroptera species can be considered as sensitive, moderately sensitive or even pollution resistant. This is particularly obvious in the Baetidae family which shows a very large range of sensitivities to organic pollution. This family is represented by 13 different species which are very useful to point out the difference of sensibility between the different species. *Baetis melanonyx*, *Alainites muticus* are very sensitive, on the contrary, *Baetis vernus*, *Baetis fuscatus*, *Baetis scambus* and *Baetis vardarensis* are more tolerant (Fig. 2).

#### Discussion

Our results show that Ephemeroptera are well distributed over the whole diagram of the CCA and that they cover all environmental conditions investigated in this study. This group is particularily sensitive to the mineralisation of the substrat. This is clearly demonstrated by the conductivity and the carbonate hardness gradient. This confirms the importance of water alkalinity in determining mayflies community composition. Another important gradient is represented by the organic pollution gradient which shows a change in mayflies composition. In rapid bio-assessment based on a family level of identifications as the IBGN (AFNOR 1992), the Baetidae family is usually considered as tolerant to organic pollution. As we have seen the example of the Baetidae family it is important to work with species-level identifications to show the difference of sensibility induced by anthropogenic disturbances. The mayflies community seems to respond to physiogeographical features as the slope, the stream order and the temperature. The slope is the second most important gradient and is also an important variable in explaining differences in species composition. The stream order and the temperature affect also mayfly distribution. This result can be understood as a confirmation of the high significance of the longitudinal zonation.

In conclusion, we can say that Ephemeroptera show many features making them a useful group for assessing aquatic biodiversity and quality in rivers. The knowledge of the distribution of the Ephemeroptera is essential in order to assess more accurately the conservation status and biodiversity of many aquatic ecosystems.

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#### References

- AFNOR., 1992. Détermination de l'indice biologique global normalisé (IBGN). Association française de normalisation (AFNOR), pp. 9.
- Cao Y., Bark A.W., 1997. A comparison of clustering methods for river benthic community analysis. Hydrobiologia, 347:25-40.
- Dohet A., 2000. Are caddisflies an ideal group for the assessment of water quality in streams? Proc. 10th Int. Symp. Trichoptera, Potsdam, Germany. 15(2001): pp.14.
- Dolisy D., 2000. Les éphémères du Grand-Duché de Luxembourg: 1. Partie rhithrale des cours d'eau: Faunistique [Ephemeroptera]. Ephemera. 2(1):7-14.
- Jackson D.A., 1993. Mutivariate analysis of benthic invertebrate communities: the implication of choosing particular data standardizations, measures of association and ordination methods. Hydrobiologia. 268:9-26.
- Leclercq L., Maquet B., 1987. Deux nouveaux indices chimique et diatomique de qualité de l'eau courante. Application au Samson et à ses affluents (Bassin de la Meuse Belge). Comparaison avec d'autres indices chimique, biocénotique et diatomique. Inst. Roy. Sci. Nat. Belg. 38:1-112.
- ter Braak C.J.F., Smilauer P., 1998. Reference manual and user's guide to Canoco for Windows: Software for Canonical Community Ordination, Microcomputer Power, Ithaca, NY, USA, pp.352.
- ter Braak C.J.F., Verdonschot P.F.M., 1995. Canonical correspondance analysis and related multivariate methods in aquatic ecology. Aquatic Sciences 57:225-289.