

The ecological requirements of caddisflies larvae (Insecta: Trichoptera) and their usefulness in water quality assessment of a river in south-west Romania

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ABSTRACT

Key-words:
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This survey analyzed the eco-physiological requirements of caddisflies assemblages in Jiu River (south-west Romania). The output revealed that the communities are driven by a synergic combination of natural factors and pollutants. Their ecological profiles are diverse and the degree of tolerance is related to a decrease of water quality over a longitudinal gradient, overlapped with natural variation of physico-chemical factors. The assemblage is dominated by hydropsychids, very tolerant to anthropogenic disturbances, and two other communities separated by large distances (characteristic of headwaters and of lowland areas, respectively) that do rather reveal stronger affinities to the microhabitat requirements than to water pollution. Polyhedral diagrams were employed in order to reveal caddisflies larvae tolerances/intolerances to environmental parameters, in order to test their usefulness for local areas and less numerous samples, as frequently encountered in routine surveys.

RÉSUMÉ

Les exigences écologiques des larves de trichoptères (Insecta : Trichoptères) et leur utilité dans l'évaluation de la qualité de l'eau dans le sud-ouest de la Roumanie

Mots-clés :
trichoptères,
rivière Jiu,
diagrammes
polyédriques,
pollution,
Roumanie

Cette étude a analysé les exigences éco-physiologiques des communautés de trichoptères dans la rivière Jiu (sud-ouest de la Roumanie). Le résultat a révélé que les communautés sont induites par des combinaisons synergiques de facteurs naturels et de polluants. Leurs profils écologiques sont divers et le degré de tolérance est lié à une diminution de la qualité de l'eau sur un gradient longitudinal, combinée à la variation naturelle des facteurs physico-chimiques. L'assemblage est dominé par des hydropsychidés, très tolérants aux perturbations d'origine anthropique, et deux autres communautés séparées par une grande distance (caractéristiques des eaux d'amont et des zones de plaine, respectivement) qui révèlent plutôt de fortes affinités avec les conditions du microhabitat, qu'à la pollution de l'eau. Des diagrammes polyédriques ont été utilisés pour mettre en évidence les tolérances / intolérances des larves de trichoptères aux paramètres environnementaux, afin de tester leur utilité pour les zones réduites et des échantillons moins nombreux, comme c'est le cas dans les suivis de routine. L'indice biotique (BI) a révélé une détérioration de la qualité de

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l'eau sur un profil longitudinal, ce qui prouve la possibilité d'utiliser un seul groupe de macro-invertébrés dans l'évaluation de la qualité de l'eau, basée sur une petite quantité de données (60) et des sites d'échantillonnage (5).

INTRODUCTION

The rivers have been massively impacted by human induced disturbances all over the world. Mainly the abiotic factors with disturbance regime determine the composition and functionality of macroinvertebrates communities (Camargo *et al.*, 2011; Resh *et al.*, 1988; Townsend *et al.*, 1997; Wildsmith *et al.*, 2011). The River Continuum Concept (Vanotte *et al.*, 1980), as the River Habitat Template (Townsend and Hildrew, 1994) relates the functional groups of macroinvertebrates to gradients of environmental stability, as their replacement on a longitudinal gradient. Such a mixture of life history strategies, habitat requirements, as the tolerance or sensitivity to pollution makes broader statements, very often outside a local scale, very difficult to interpret (Hildrew, 1986; MacCausland and McTammany, 2007; Useggio-Polattera *et al.*, 2000).

The caddisflies represent one of the most widely used macroinvertebrates in river quality assessments, along with the mayflies and stoneflies, and the most up to date statistical packages are using them as one of the main focus groups (e.g. RIVPACS, AusRivAS) (Castillo *et al.*, 2006; Shiels, 2010; Wright *et al.*, 2000). One of the main reasons for their usefulness can be found in the extremely widespread distribution in every type of aquatic habitat, as in their noticeable frequency amid freshwater insects (Basaguren and Orive, 1990; Illie and Botosăneanu, 1963; Mackay, 1984).

Nevertheless, the attempt of classifying the water quality of a stream based solely on a single group of invertebrates was approached so far only erratically and by only a handful of studies (Bonada *et al.* 2004; Dohet, 2002; Fairchild and Holomezki, 2002; Resh, 1992). Previous investigations employed large data sets in order to counterbalance many inconveniences encountered during routine surveys, e.g. extrapolations for wider geographical and geological areas (Bonada *et al.*, 2005) that could concur and overlap with natural induces regimes. The investigation of the autecological requirements of caddisflies from a longitudinal perspective in lotic ecosystems, and more important, their use as a single group in the attempt to test their usefulness as indicators of water quality was never approached on the Romanian territory. The caddisflies have been described previously as an ideal group for freshwater impact assessments, and this survey aims in describing the eco-physiologic requirements of caddisflies larvae in a stream impacted by increasing anthropogenic pressures from the headwaters towards lowland areas.

Therefore, the testing hypothesis is that the community of caddisflies will comprise different assemblages as a result of a synergic impact between the variation of natural variables over a longitudinal profile and their overlap with pollutants. Therefore, we predict that identifiable abiotic factors with the help of mainly descriptive multivariate statistics will perform as good predictors in establishing the range of environmental parameters, as of control factors of the caddisflies presence/absence along the longitudinal transect of the studied river.

MATERIALS AND METHODS

The River Jiu lies in the southwest of Romania and represents an important tributary of the Danube. Historically, the entire catchment used to be exploited for charcoal, the mining areas comprising a large surface of the actual drainage basin. Nowadays, the mining activities ceased only partially, with remnant ash pits still visible a few meters further away from the main stem of the river, amplified by a few still active mining points. This, and several water works, overlapped with the activity of sewage treatment plants and industrial centres

Table II

Mean and standard error of the mean ($1 \pm SE$) for the physico-chemical parameters measured on each site.

Physico-chemical parameters	S1	S2	S3	S4	S5
Suspended sediment (mg·L⁻¹)	53.2 ± 0.8	33.7 ± 0.6	44.20 ± 0.9	54.5 ± 0.9	20.5 ± 0.4
SO₄ (mg·S·L⁻¹)	49.2 ± 2.7	31.2 ± 2.1	35.4 ± 2.3	50.3 ± 0.7	25.8 ± 0.6
Conductivity (µS·L⁻¹)	191.5 ± 17.1	185.8 ± 9.9	167 ± 8.7	99.2 ± 3.7	96.8 ± 1.4
BOD₅ (mg·L⁻¹)	3.6 ± 0.3	4.4 ± 0.5	4.7 ± 0.7	1.1 ± 0.1	1.5 ± 0.07
Total Organic Carbon (mg·L⁻¹)	1.58 ± 0.8	1.07 ± 1.4	1.06 ± 2.9	0.8 ± 0.4	0.25 ± 0.5
Alkalinity (mg·L⁻¹)	1.3 ± 0.01	1 ± 0.06	0.9 ± 0.05	0.8 ± 0.02	0.9 ± 0.1
NH₄ (mg·N·L⁻¹)	0.15 ± 0.007	0.11 ± 0.02	0.13 ± 0.008	0.15 ± 0.03	0.04 ± 0.01
NO₂ (mg·N·L⁻¹)	0.08 ± 0.01	0.08 ± 0.01	0.1 ± 0.01	0.006 ± 0.0009	0.03 ± 0.009
NO₃ (mg·N·L⁻¹)	2.9 ± 0.3	3.2 ± 0.3	1.8 ± 0.34	1.02 ± 0.12	0.4 ± 0.11
PO₄ (mg·P·L⁻¹)	0.08 ± 0.01	0.11 ± 0.01	0.15 ± 0.03	0.004 ± 0.0004	0.01 ± 0.001
Water temperature (°C)	10.2 ± 1.7	10.7 ± 1.8	9.3 ± 1.4	10.1 ± 1.78	6.9 ± 1.9
Oxygen (% saturation)	85% ± 0.02	6% ± 0.02	10% ± 0.04	0.9 ± 0.03	134% ± 0.17
Water flow (m·s⁻¹)	7.4 ± 0.3	7 ± 0.33	9.8 ± 0.3	8.4 ± 0.5	10.6 ± 0.1
pH	8.32 ± 0.12	7.5 ± 0.06	8.3 ± 0.07	7.9 ± 0.1	7

influences of local sewage treatment plants. The site S₂ was chosen after the confluence with a cleaner tributary (River Tismana) and the S₁, the further away site, downstream the City of Rovinari, in order to illustrate one of the most degraded river stretches from this watershed due the combination of aforementioned human induced disturbances (Dumbravă-Dodoacă and Petrovici, 2010). On each visit were taken three replicates of biological, physico-chemical measurements as of water samples, resulting altogether in 60 replicates of each kind. The biological samples were preserved in 8% formaldehyde. The invertebrates were collected from every site in three replicates with a Surber sampler (surface 1073 cm² and 250 µm mesh size) and their abundance was estimated/m². The water temperature, dissolved oxygen, conductivity, pH and flowing velocity were measured *in situ*, with a specialized probe (350i SET type multimeter). Three recipients of water samples (each of 1 l volume) were sent to the local laboratory for water quality in order to be analyzed for: suspended sediments in the water column, sulphates, biological oxygen demand (BOD₅), total organic matter as free dried ash (TOC), alkalinity, N species (nitrites, nitrates, ammonia) and phosphate concentrations (Table II). The caddisflies were sorted in the laboratory with the aid of a dissecting microscope and identified afterwards to species level (Waringer and Graf, 1997; Wallace *et al.*, 2003).

Each site comprised one riffle with similar dimensions: 18.3 ± 2.31 m wide, respectively 0.28 ± 0.08 m deep in average. The type of substratum varied from boulders up to sand and ooze (personal observations).

Apart from pH, all the physico-chemical parameters were $\log(x + 1)$ (Table II) and tested for normality (Kolmogorov-Smirnov test). After the assumptions of normality were met, a PCA analysis was applied in order to reveal the variance amid sampling sites, and the eigenvalues of the first two axes, along with their percentage of variance were stated and checked for eligibility, according to the "broken stick" model (Shaw, 2003) (Table III) (Figure 2).

The caddisflies densities were $\log(x + 5)$, checked for normality and the relationship between their abundance and environmental parameters estimated with the help of pCCA (partial Canonical Correspondence Analysis) (CANOCO 4.5 for Windows). The significance of

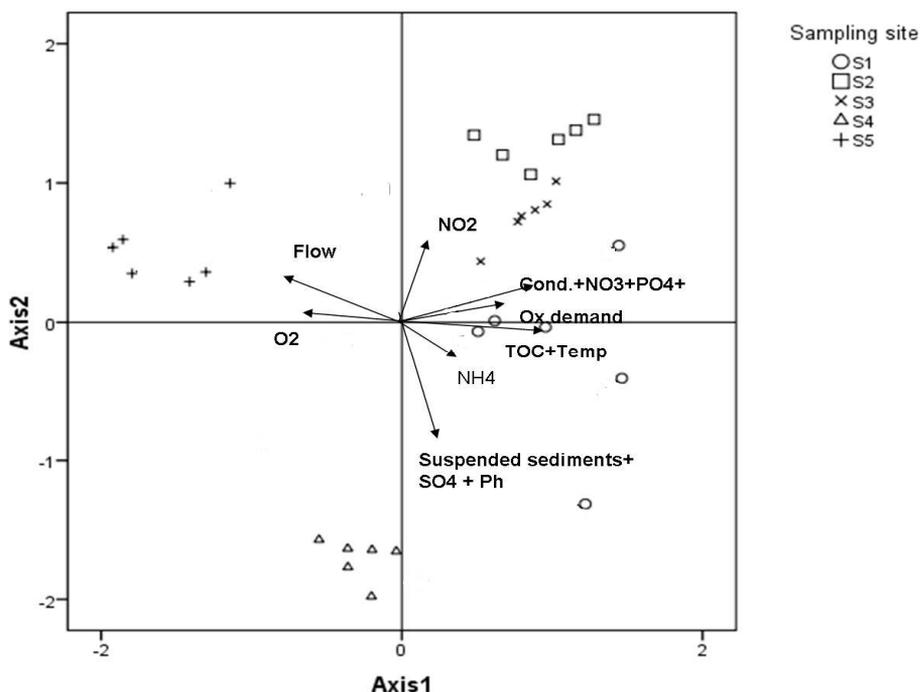


Figure 2

PCA ordination of physico-chemical parameters along sampling sites. The physico-chemical variables are analysed along an upstream-downstream gradient along the first axis (water velocity, dissolved oxygen, conductivity, nitrates, phosphates, total organic carbon, temperature and biological oxygen demand). Along the second axis there is a variation between sampling sites S2 and S4 (nitrites, suspended sediments, sulphates and pH).

Table III

Eigenvalues, explained and cumulative percentages explained by the first two axes of the PCA ordination.

	Eigenvalues	Explained percentage	Cumulative percentage (%)
Axis 1	5.7	40.7	40.7
Axis 2	2.44	17.5	58.3

environmental parameters in explaining the existence of taxa was checked upon a Monte Carlo test (999 permutations) (Shaw, 2003). Due the overlap and collinearity between natural and human induced variability in the environmental data sets, two partial CCA were needed (Figures 3 and 4). After selecting the appropriate environmental variables, a stepwise linear regression was applied for each species in order to retain the variables that are the best predictors in explaining their abundance. A TWINSPLAN analysis (DECORANA program), was applied in order to retain those taxa that could be used as “indicator” species (Shaw, 2003) (Figure 5).

A more qualitative approach, based on weighted means was applied, following the recommendations of Bonada *et al.* (2004), in order to see if the findings from larger data sets are applicable for more local areas and smaller data sets. Therefore, following the recommendations of ter Braak and Barendregt (1986) and those of Birks *et al.* (1990), an optimum and tolerance values were calculated for each taxa considering those variables found as significant after the application of the liner regression models. The values for tolerances/each taxa were standardized to values below unit, by dividing them to the highest calculated value (Bonada *et al.*, 2004). Afterwards, a polyhedral diagram (Figures 6a–6c) was constructed by orientating the tolerance values for the selected environmental parameters towards a common point at equal angles, summing altogether 180°. The parameters related to pollution

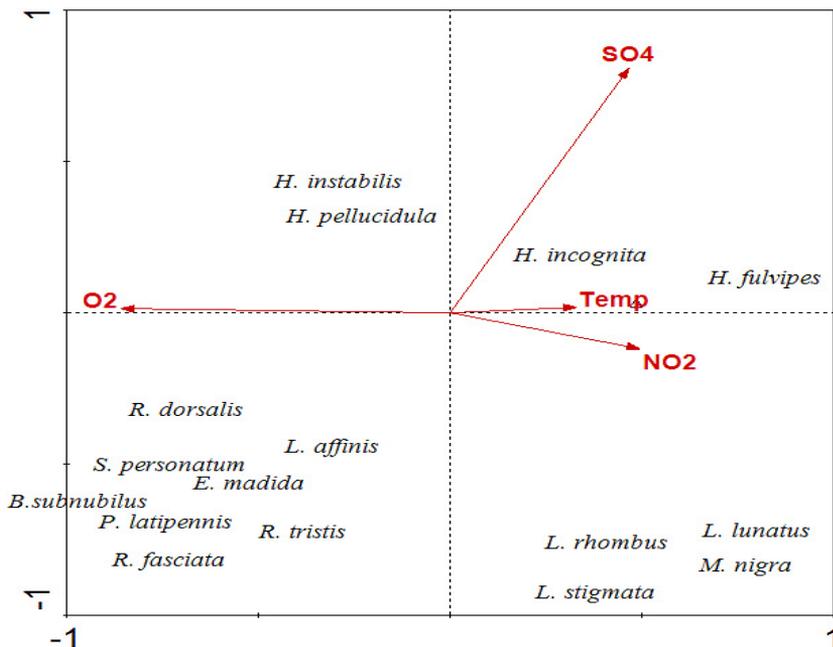


Figure 3
 Partial CCA ordination plot of caddisflies species mainly as a result of chemical pollution. The natural decrease from headwaters to lowland sites of temperature and oxygen is depicted along the first axis, revealing a preference for different groups of caddisflies. The variation of sulphates along the second axis separates common hydrosychids from other two species confined more downstream.

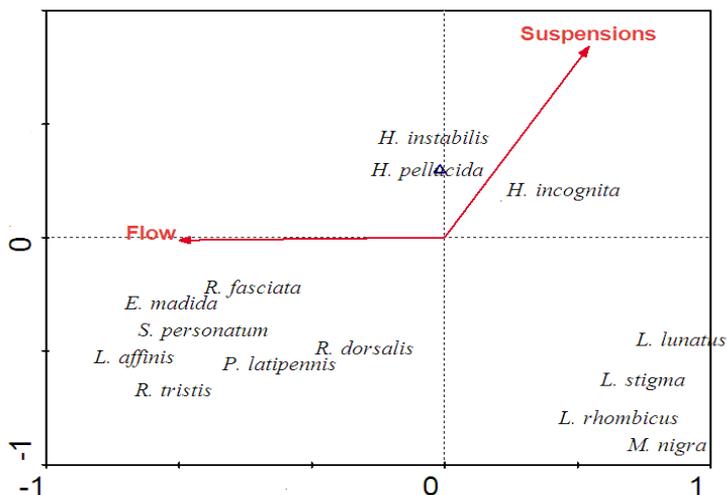


Figure 4
 Partial CCA based only on water velocity and suspended sediments in water column. The water velocity decreases along the river longitudinal profile (first axis), with same groups as above (Figure 3) separated by a long distance. The hydrosychids reveal a preference for high suspended organic loads (second axis).

(sulphates, nitrites, phosphates and oxygen demand-CBO₅) were oriented with their origins towards the outer side of the diagram. The variables related to pristine conditions (dissolved oxygen, water velocity) were drawn from 0 to 1 inside (Bonada *et al.*, 2004). The degree of tolerance/intolerance was estimated at a qualitative level by unifying the upper and lower limits of tolerances and colouring the unified area. The shadowed areas represent the degree of tolerance and the white spaces (area within the diagram) the degree of intolerance. In some cases, because of the large value of the lower/ upper limits, there are non shaded

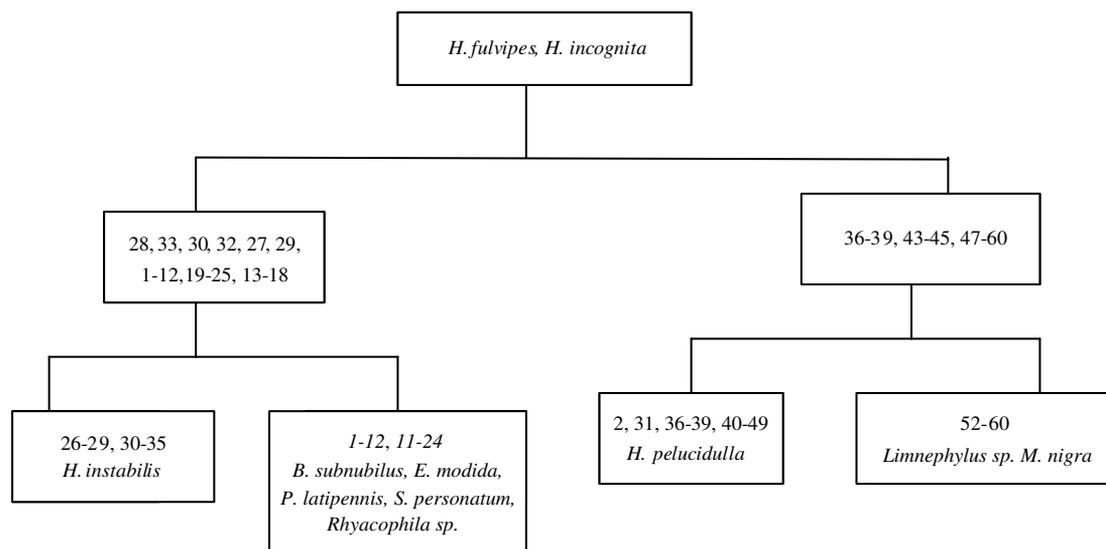


Figure 5
TWINSpan dendrogram clusters of taxa (comments in text).

areas, indicating that the taxa should be infrequent in values below/above these conditions. The diagrams were clustered in groups, according to the clades separated by the TWINSpan dendrogram (Figure 5).

RESULTS

The first axis of the PCA ordination (Figure 2) is a consequence of an altitudinal transect from the headwaters (S_5) towards the downstream sites, and represents a decrease of annual mean water velocity, dissolved oxygen, and an increase in temperature, associated refractory organic matter, oxygen demand, alkalinity, conductivity, N-compounds (nitrites, nitrates, ammonia) and phosphates as a direct consequence of pollution, as revealed by their means across sampling sites (Table II). The second axis is mainly an effect of nitrites (higher in S_2) and higher concentration of sulphates, suspended solids and pH for S_4 . Therefore, the heterogeneity of the water physic-chemistry along the sites is reflected along the both axes as a potential suite of “disturbances” along a longitudinal path. Both axes explain a variance of 58.3% of physic-chemicals between sites (Table III), the main axis explaining 40.7%.

The partial CCA (pCCA) ordination plot of caddisflies larvae and chemical parameters (Figure 3) illustrates the factors found eligible in explaining the variability of taxa: the dissolved oxygen, temperature, nitrites and sulphates in a proportion of 70% (Monte Carlo test, 999 permutations). The caddisflies reflect a strong affinity for high and low altitude sites, as for pristine and polluted areas of the river. The species from genus *Rhyacophila* sp., *Sericostoma personatum*, *Eclisopteryx madida*, *Potamophylax latipennis*, *Limnephilus affinis* have a clear affinity for highly oxygenated and pristine headwaters. The species from genus *Hydropsyche* sp. are generalist taxa that have a high tolerance for high levels of sulphates and a third group, represented by species from genera *Limnephylus* sp. and *Mistacydes nigra* as being characteristic for lowland, warmer, polluted sites, with seemingly a greater tolerance for nitrites. Amid the species from the genus *Hydropsyche* sp., there is a separation along the second axis between species that have a lower tolerances to decreased oxygen concentrations, like *H. instabilis* and *H. pelucidulla* as compared to *H. fulvipes* and *H. incognita* (Figure 3).

Due the high co-correlations between water pollutants and the natural selection of sites over an altitudinal gradient (and therefore the natural occurrence and variability of certain factors like water flow, oxygen and temperature), a second partial CCA was needed (Figure 4). The

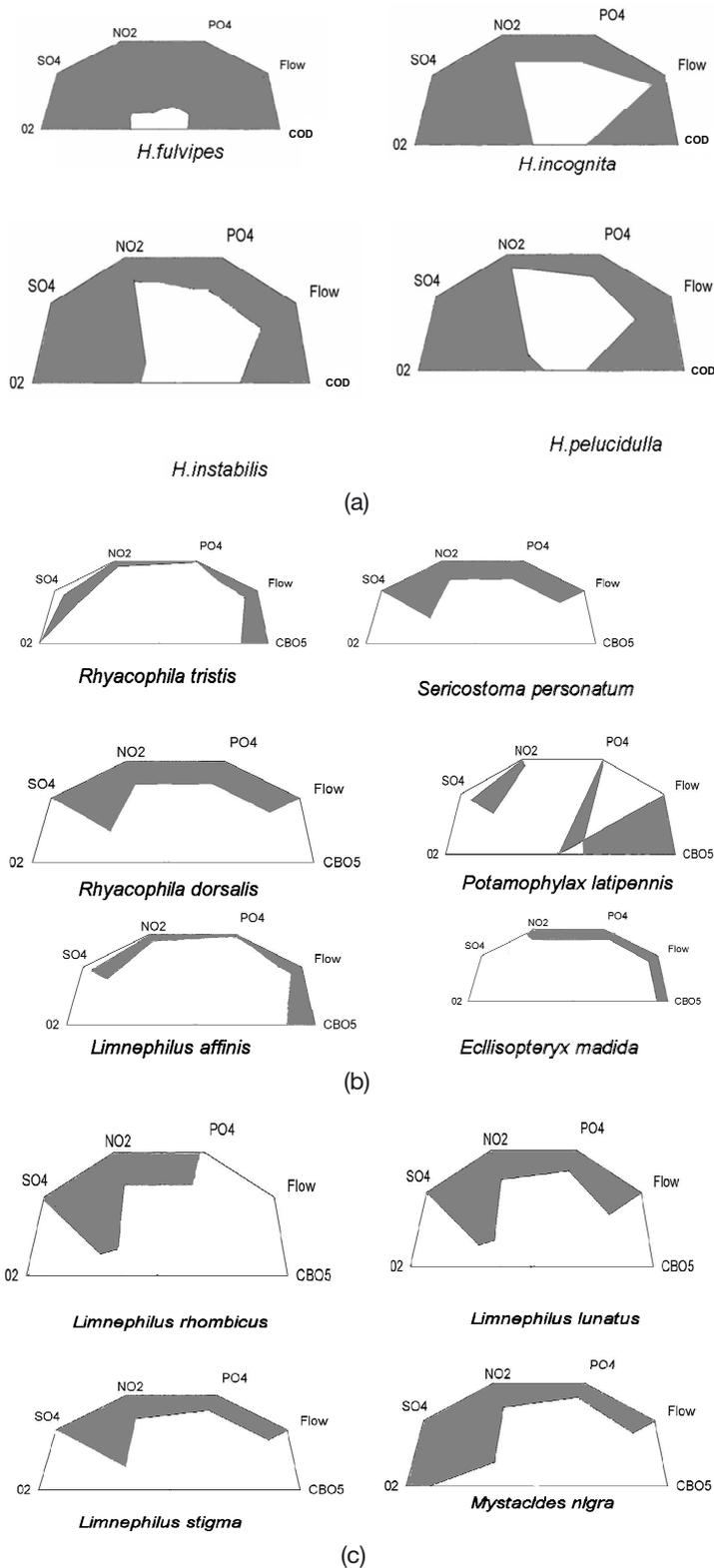


Figure 6

(a) Ecological profiles for common caddisflies based on polyhedral diagrams (Family Hydropsychidae).
 (b) Ecological profiles for caddisflies characteristic to headwaters, based on polyhedral diagrams.
 (c) Ecological profile of caddisflies species characteristic for lowland sites, based on polyhedral diagrams.

Table IV

Linear regression equations for the caddisflies species and physico-chemical variables selected by the Monte-Carlo test from the partial CCA ordinations. The step wise linear regression was applied to $\log(x + 1)$ biotic and abiotic factors.

Taxa	Equations	Adjusted R^2	F	P
<i>H. fulvipes</i>	$135 - 0.65 \times \log(\text{Flow} + 1) + 0.24 \times \log(\text{PO}_4 + 1)$	0.55	38.01	<0.001
<i>H. incognita</i>	$-117.1 + 0.38 \times \log(\text{CBO}_5 + 1) + 0.37 \times \text{pH}$	0.35	15.6	<0.001
<i>H. instabilis</i>	$-79 + 0.4 \times \log(\text{O}_2 + 1) + 0.4 \times \log(\text{SO}_4 + 1)$	0.29	11.03	<0.001
<i>H. pelucidulla</i>	$-3.3 + 0.3 \times \log(\text{O}_2 + 1) - 0.04 \times \log(\text{SO}_4 + 1)$	0.12	3.69	0.03
<i>E. modida</i>	$-0.4 + 0.5 \times \log(\text{O}_2 + 1) - 0.28 \times \log(\text{SO}_4 + 1)$	0.37	17.13	<0.001
<i>L. affinis</i>	$0.84 + 0.6 \times \log(\text{O}_2 + 1) - 0.46 \times \log(\text{SO}_4 + 1) - 0.25 \times \log(\text{NO}_2 + 1)$	0.71	47.6	<0.001
<i>P. latipennis</i>	$-0.77 + 0.5 \times \log(\text{O}_2 + 1) - 0.35 \times \log(\text{SO}_4 + 1)$	0.44	22.6	<0.001
<i>R. dorsalis</i>	$-1.9 + 0.58 \times \log(\text{O}_2 + 1) - 0.35 \times \log(\text{SO}_4 + 1)$	0.58	39.77	<0.001
<i>R. fasciata</i>	$0.25 + 0.48 \times \log(\text{O}_2 + 1) - 0.42 \times \log(\text{SO}_4 + 1) - 0.2 \times \log(\text{NO}_2 + 1)$	0.5	18.7	<0.001
<i>R. tristis</i>	$-0.17 + 0.55 \times \log(\text{O}_2 + 1) - 0.34 \times \log(\text{SO}_4 + 1) - 0.13 \times \log(\text{NO}_2 + 1)$	0.49	18	<0.001
<i>R. personatum</i>	$-6.3 + 0.65 \times \log(\text{O}_2 + 1) - 0.4 \times \log(\text{SO}_4 + 1)$	0.65	6.4	<0.001
<i>L. rhombicus</i>	$2.7 - 0.5 \times \log(\text{O}_2 + 1) + 0.23 \times \log(\text{Temp} + 1)$	0.34	14.8	<0.001
<i>L. lunatus</i>	$1.9 - 0.54 \times \log(\text{O}_2 + 1) + 0.3 \times \log(\text{Temp} + 1)$	0.32	13.72	<0.001
<i>L. stigma</i>	$3.4 - 0.54 \times \log(\text{O}_2 + 1) + 0.22 \times \log(\text{Temp} + 1)$	0.37	17	<0.001
<i>M. nigra</i>	$1.01 - 0.51 \times \log(\text{O}_2 + 1) + 0.2 \times \log(\text{Temp} + 1)$	0.33	13.98	<0.001

pCCA run on only two environmental parameters (water velocity and solid suspensions) do explain 94% from the variability of taxa (Flow – $F = 33.5$, $p = 0.002$, respectively solid suspensions – $F = 40.3$, $p = 0.002$, Monte Carlo test, 999 permutations) between sites. The ordination diagram reflects the preferences of three main groups of caddisflies: the first axis is explained by the water flow decreasing over an altitudinal transect, and with species characteristic from the genera *Rhyacophila* sp., *S. personatum*, *P. latipennis* and *E. madida* as having a preference for headwaters, with a higher water flow, highly oxygenated and low turbidity. The other group, represented by species from the genera *Limnephilus* sp. and *M. nigra*, provides a preference for lower altitude sites (e.g. more lentic like environments). The second axis is mainly a function of suspended solids, with the species from genus *Hydropshyche* sp. as having a preference for high water turbidity.

The linear regression equations (Table IV) reflect a better understanding in the attempt to find a quantitative relation amid their densities and the variability of environmental parameters. The stepwise linear regression method was employed, discarding the strong colinearity between factors and retaining only those that explain (in a both positive and negative way) the taxa densities with the highest strength.

The factors selected for the polyhedral diagrams (Figures 6a–6c) were selected from those having a significant influence on the caddisflies abundance in the multiple regression equations and not from those represented in the pCCA 's, due criteria like colinearity and

overlapping ecological significance for explaining the existence of taxa. Therefore, the elements selected in order to explain the species status as being sensitive or tolerant organisms, were the sulphates, nitrites, phosphates and biological oxygen demand (BOD₅) (as factors directly related to pollution) and dissolved oxygen and water velocity (as factors related to pristine conditions).

The TWINSpan dendrogram (Figure 5), based on pseudospecies frequencies, selected three main groups: those common in all sites (*H. fulvipes* and *H. incognita*), as two other species from the same genus, but absent from the most downstream sites (Figure 3). The second dichotomy is based on the clustering of two completely different assemblages: those characteristic to the pristine headwaters (*Rhyacophila* sp., *B. subnubilus*, *S. personatum*, *P. latipennis*, *L. affinis*) and those characteristic to downstream sites, more lentic and more tolerant to pollution (*Limnephilus* sp., *M. nigra*) (Figures 3, 4).

DISCUSSION

The TWINSpan analysis separated three groups: a common one, representative by four species from genus *Hydropsyche* sp. Two of them (*H. fulvipes* and *H. incognita*) are present in all sampling sites, all year round. As stated in Figure 6a, these two species seem to be the most tolerant taxa to water pollution and variability of physico-chemical parameters. Nevertheless, the other two species from the same genus (*H. instabilis* and *H. pellucidula*), seem to be separated mainly as a function of dissolved oxygen, being absent in the downstream sites. This finding is in accordance to Becker (1987), who found that the latter species is sensitive to low dissolved oxygen concentrations, and more characteristic to moderate polluted stretches of Rhine River. Bonada *et al.* (2004) suggested that *H. instabilis* is intolerant to low concentrations of dissolved oxygen. According to the regression equations, and separation by TWINSpan dendrogram (Figure 5), the other two species of caddisflies are explained by different factors (e.g. sulphates) although their tolerances are not that relevant on the polyhedral diagrams (Figure 6a). Bonada *et al.* (2004) found that the species *H. instabilis* is intolerant to high concentrations of sulphates.

Previous studies have reported different behaviours of species from genus *Hydropsyche* sp. to environmental changes as for their tolerances to the variability of the natural physico-chemical parameters along the longitudinal profile of streams (Bonada *et al.* 2004; Ruse and Herrmann, 2000; Garcia *et al.*, 2006). Moreover, the species from this genus have been showed as revealing preferences for certain lotic microhabitats, with a strong interspecific competition for food and space, and even a replacement of the taxa on a longitudinal profile of rivers (Edington, 1968; Hilldrew and Edington, 1979; Wallace and Merritt, 1980). The species *H. pellucidula* and *H. siltalai* were found as coexisting in British streams, same for *H. instabilis* and *H. siltalai* (Hilldrew and Edington, 1979), their positions being explained by preferences for certain regimes of water temperature, flow, food, oxygen and pollution. Nevertheless, these species co-exist in the same riffles, revealing a strong interspecific tolerance with regard to competition for food and space. All the species from the genus *Hydropsyche* sp. were found as having a strong affinity for suspended sediments in water column, in accordance to previous studies (Basaguren and Orive, 1990; Mackay, 1980). This is mainly due their filter feeding behaviour, with nets capturing flowing organic matter in suspensions with different dimensions (Wallace and Merritt, 1980).

The headwaters were populated by a completely different suite of taxa as compared to the downstream sites, with three species from the genus *Rhyacophila* sp. characteristic only for the headwaters. The species *R. tristis* was found as being characteristic to the headwaters of Spanish rivers (Basaguren and Olive, 1990), as for English streams (Edington and Hilldrew, 1981) and being more sensitive than *R. dorsalis* to environmental pollution (Bonada *et al.*, 2004), in accordance to our observations (Figure 6b). This latter species was found in headwaters and midstream with contrasting water qualities (Bonada *et al.*, 2005), although in the polluted sites neither of these species being found, suggesting therefore a strong sensibility to high water quality. The coexistence of all three species of *Rhyacophila* sp.

could be explained by a prey resource partitioning as suggested for sympatric caddisflies larvae previously (Malas and Wallace, 1977; Teague *et al.*, 1985; Alstad, 1987). The species *B. subnubilus* is a univoltine species, generally found in low abundances, but representing an important percentage in the rivers secondary production and prefers moderate to high cold water flow stretches, well oxygenated and extremely sensitive to suspended sediments (Gunn, 1987; Anderwald, 1996). *S. personatum* is a shredder, extremely important in the leaf litter energy transfer from lower to higher trophic levels, and its presence and previous studies have regarded this taxa mainly from this point of view (Frieberg and Jacobsen, 1999; Iversen, 1974, 1979). Bonada *et al.* (2004) found this genus as sensitive to water pollution, in accordance to our observations. *P. latipennis* and *E. madida*, along with *L. affinis* are limnephilids that provide a strong intolerance to water pollution (Figure 6b). In what concerns *P. latipennis*, our observations are in accordance to those of Bonada *et al.* (2004), who described this taxa as one characteristic for pristine river stretches, intolerant for most of the inorganic pollutants, including heavy metals (Vuori, 1993). Bonada *et al.* (2005) found this latter species as being a rare one in the trichopteran assemblages from Spanish rivers, a common situation in this survey, and same for *B. subnubilus*. *L. affinis* is a species that was found in this survey only in the headwaters, although its relatives from the same genus were found confined further downstream. This species was found as having a strong dependency of life cycle to the ambient temperature (Gislason, 1978) and its presence could be explained by preferences for colder, highly oxygenated river stretches, like for the headwaters of this river.

The third group, comprising species appurtenant to genus *Limnephilus* sp. and *M. nigra* (Family Leptoceridae) were found only in S₂, after the confluence with a much cleaner tributary, the Tismana River (Figure 1). The pCCA suggest this group as being characteristic to lowland sites (Figure 4), with a lower water velocity and much warmer than the headwaters, low suspended sediments and high tolerance to nitrites. However, the tolerance for nitrites is not significantly different as compared to the limnephilids and *B. subnubilus* tolerance levels from headwaters, and therefore not noticeable as well on the polyhedral diagrams (Figure 6c). The regression equations explain the density of these taxa as mainly a function of oxygenated waters (negatively) and higher temperatures (positively). Considering that these taxa were found only in this site (S₂), after the confluence with a cleaner tributary, it could be reasonable to explain the lack of tolerances for higher concentrations of pollutants, but rather a preference for lower oxygen and higher temperature than those registered in the upstream sites on the grounds of their spatial location. The cleaner waters of Tismana River are providing better conditions for the existence of this community and explain its absence from more downstream sites (S₁), where only hydroptychids was found. Therefore, the results suggest that this community of caddisflies is rather influenced by natural variance of physic-chemical factors (e.g. preference for more lentic waters and higher temperature) than rather by the increase in pollutants concentrations and as being massively influenced by the confluence of the main stem of polluted Jiu River with the cleaner tributary of Tismana River.

As stated in previous studies (Anderson and Klubnes, 1983; Basaguren and Olive, 1990; Boon, 1979; Edington, 1968; Hilldrew and Edington, 1979) the longitudinal habitat partitioning, different eco-physiological requirements, inter- and intraspecific competition for food and space are important drivers in species co-existence and/or replacement of taxa from headwaters toward lowlands.

CONCLUSIONS

The investigated species of caddisflies from the River Jiu seem to be influenced by a synergic combination of natural physic-chemical parameters (e.g. decrease of dissolved oxygen, water velocity, increase of suspended solids and temperature) and pollutants (N species, phosphates, sulphates, BOD₅, TOC, conductivity) on a longitudinal transect. One of the three depicted "clusters" may actually comprise the dominant and most frequent one group, the hydroptychids, that are very widespread and tolerant to both natural and human induces disturbances. The other two clades, separated by a long distance, and inhabiting habitats with

different characteristics, might actually comprise two groups of caddisflies who are not that different with respect to their toleration to pollutants, but rather employing different habitat requirements. The positioning of the latter group is perhaps massively influenced by the junction of the polluted main stem of River Jiu with the cleaner tributary of Tismana River.

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