

## Leeches (Clitellata: Hirudinida) of an upland stream: taxonomic composition in relation to habitat conditions

by

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### Abstract

Hirudinida comprise a constant component of benthofauna in running waters and their taxonomic composition is often analyzed to evaluate the environmental quality. However, the indicative role of particular species is still arguable. Thus, it is very important to provide more detailed information on the impact of environmental parameters on the species structure within this group of invertebrates. Representatives of nine species were found in the investigated upland stream including mainly *Erpobdella vilnensis* and *E. octoculata*. The research has demonstrated that the hydromorphological conditions and feeding behavior are the main factors determining the taxonomic composition of leeches in particular sections of a watercourse.

**Key words:** Hirudinida, stream, environmental relationships, water pollution

## Introduction

Leeches are a group of mostly aquatic organisms, consisting of about 680 species (Sket, Trontelj 2008), including 47 species occurring in central Europe (Bielecki et al. 2011). They are an important element of aquatic ecosystems and often regarded as environmental indicators. However, the knowledge about habitat preferences and tolerance of particular species still does not correspond to the indicative role assigned to these animals in monitoring research.

Many authors have provided extensive information on the significance of physicochemical parameters of water and the substrate type for the distribution of leeches (Pawłowski 1936; Agapow 1980; Sawyer 1986; Brönmark 1992; Bielecki et al. 2004, 2008; Koperski 2010; Beracko, Kosel 2011). However, attempts at indisputable confirmation of the role and significance of particular environmental factors often end in failure. So far little attention has been paid to the impact of the catchment type and canopy cover on leech assemblages in streams (Koperski 2010, Kubova et al. 2013). Whereas the catchment character may considerably modify the habitat parameters in watercourses. In streams heavily affected by agricultural or urban land uses, nutrient concentrations and conductivity could be elevated, while oxygen concentrations reduced. All these factors, changing seasonally or even in the daily cycle, can considerably affect the composition of macroinvertebrate assemblages, including leeches.

Despite the fact that there are many papers on the ecology of leeches, accurate knowledge about factors determining the habitat preferences of these animals is necessary. Moreover, previous views on the relationship between individual species and specific environmental conditions are currently under discussion (Koperski 2005). Therefore, both species composition of leech assemblages as well as the distribution and ecology of individual species in relation to habitat specificity and habitat quality should be further researched. The aim of the study was to determine the relation between selected characteristics of water quality and the catchment and species composition of leeches in an upland stream. A small watercourse was selected for this study, with the water quality and the type of catchment varied in a characteristic way. We put forward the hypothesis that the species composition of leech

assemblages reflects changes in hydromorphological and physicochemical parameters in the longitudinal profile of the stream affected by different forms of anthropic impact.

We put forward the hypothesis that the species composition of leech assemblages fluctuates along a longitudinal profile together with changes in the habitat conditions of a watercourse.

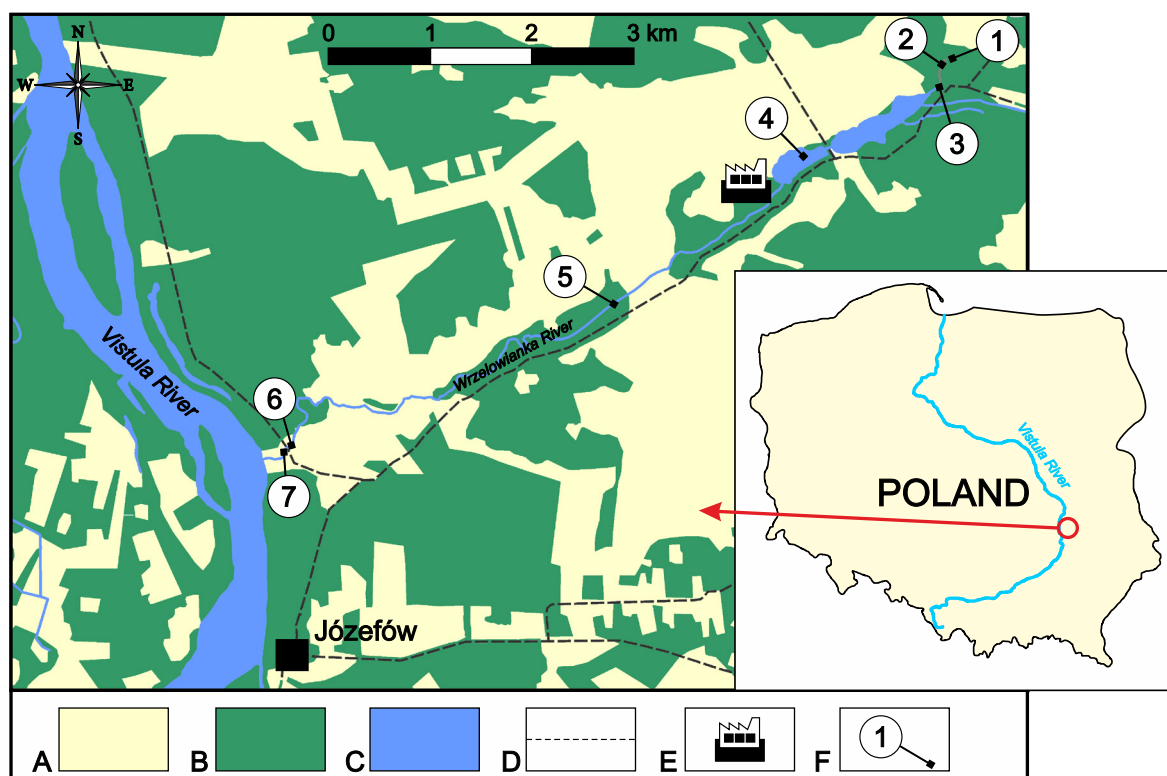
## Materials and methods

### Study area and sites

The research was conducted in the Wrzelowianka Stream, which is 9.4 km long, originates in Wrzelowiec (175 m a. s. l.) and flows into the Vistula River near Kolczyn (134 m a. s. l.). It is a single stream in a dry area furrowed with deep valleys, and no tributary downstream. Wrzelowiec (Michalczyk, Wilgat 1998; Michalczyk et al. 2008). The Wrzelowianka Stream runs in the southwestern part of the Lublin Upland, the mesoregion of Urzędowskie Heights. A chalk substrate is covered by a thick layer of loess (Kondracki 2002). The stream flows through a valley among 200-220 m. (m a. s. l.) hills with slopes planted with mixed forests (*Quercus roboris-Pinetum*) and deciduous forests (*Tilio-Carpinetum*) (Święś 1992). The research was carried out at seven sites (Fig. 1) which differ in the type of catchment and the degree of water pollution (Table 1). The bottom of the valley is partially afforested, for either agricultural purposes (meadows, cereal cultivation, fruit plantations) or to be left fallow. The stream flows through the villages of Wrzelowiec and Wólka Kolczyńska. The upper part of the stream course consists of an extensive complex of polytrophic fish ponds, and downstream of the village of Kluczkowice, a fruit and vegetable processing plant is located.

### Field studies

The faunistic material was collected from May to October 2012 using a dredge with a square frame, a grip edge of 0.25 m and mesh of 250  $\mu$ m. Samples were collected at a distance of 4 m, giving a total collecting area of approximately 1.0 m<sup>2</sup>. The collected organisms were placed into nylon bags while still in the field and then segregated in a laboratory

**Figure 1**

The study area and the conditions of the study sites. A – open area, B – forest, C – surface waters, D – main roads, E – the processing plant in Kluczkowice, F – study sites

**Table 1**

Analyzed properties of the study sites (numeration as in the main text and in Fig. 1). Measurable factors: TEM – temperature [°C], potential of acidity [pH], DO% – dissolved oxygen [%], DOppm – dissolved oxygen [mg dm<sup>-3</sup>], EC – electrical conductivity [μS cm<sup>-2</sup>], TDS – total dissolved solids [mg dm<sup>-3</sup>], SAL – salinity [PSU], CUR – current velocity [m s<sup>-2</sup>], DEP – maximum depth [m], KM – distance from the source [km]. Non-measurable factors: POL – water pollution, CAT – catchment, VEG – vegetation (codes like in the section Methods and material – Statistical analysis)

|                      | Locality number |             |             |             |             |             |             |
|----------------------|-----------------|-------------|-------------|-------------|-------------|-------------|-------------|
|                      | 1               | 2           | 3           | 4           | 5           | 6           | 7           |
| measurable factors   |                 |             |             |             |             |             |             |
| TEM                  | 10.5 ± 1.1      | 9.8 ± 0.2   | 11.8 ± 1.7  | 19.1 ± 6.3  | 20.1 ± 5.18 | 17.0 ± 5.7  | 16.0 ± 4.0  |
| pH                   | 7.96 ± 0.31     | 7.69 ± 0.41 | 8.48 ± 0.88 | 8.62 ± 0.62 | 8.51 ± 0.41 | 8.94 ± 0.65 | 8.40 ± 0.17 |
| DO%                  | 41.5 ± 15.4     | 40.7 ± 21.8 | 61.4 ± 36.1 | 64.4 ± 41.5 | 52.2 ± 23.9 | 56.5 ± 25.6 | 54.0 ± 28.1 |
| DOppm                | 4.47 ± 2.73     | 4.47 ± 2.38 | 6.34 ± 3.62 | 5.47 ± 3.10 | 4.53 ± 2.06 | 5.25 ± 2.41 | 5.12 ± 2.65 |
| EC                   | 425 ± 22        | 425 ± 20    | 429 ± 24    | 353 ± 111   | 321 ± 77    | 412 ± 91    | 507 ± 93    |
| TDS                  | 212 ± 11        | 212 ± 10    | 214 ± 12    | 176 ± 55    | 160 ± 39    | 206 ± 45    | 253 ± 46    |
| SAL                  | 0.20 ± 0.01     | 0.20 ± 0.01 | 0.21 ± 0.01 | 0.17 ± 0.05 | 0.15 ± 0.04 | 0.20 ± 0.05 | 0.24 ± 0.05 |
| CUR                  | –               | 0.43 ± 0.04 | 0.31 ± 0.08 | –           | 0.21 ± 0.05 | 0.24 ± 0.06 | 0.35 ± 0.05 |
| DEP                  | 0.30            | 0.20        | 0.40        | 2.0         | 0.60        | 0.20        | 0.40        |
| KM                   | 0.0             | 0.1         | 0.4         | 1.6         | 4.9         | 9.1         | 9.1         |
| immeasurable factors |                 |             |             |             |             |             |             |
| POL                  | 0               | 0           | 0           | 1           | 1           | 2           | 2           |
| CAT                  | 0               | 0           | 1           | 0           | 1           | 2           | 2           |
| VEG                  | 1               | 0           | 2           | 2           | 1           | 1           | 1           |

and preserved in 70% ethyl alcohol. Leeches were identified according to the identification keys by Bielecki et al. (2011) and Bielecki (2013).

A Hanna Instruments HI 9829 device was used to measure selected indicators of physical and chemical properties of water: temperature, pH, conductivity, dissolved oxygen, oxygen saturation of water, the amount of dissolved solids and salinity. Moreover, the current velocity was measured with the float method at a distance of 10 m by performing three measurements and calculation of the arithmetic mean.

### Statistical analysis

Standard deviations, Shapiro-Wilk normality test, Spearman correlation ( $r_s$ ) and Kruskal-Wallis test (H) were calculated using the Statistica 10.0 program. Species composition of Hirudinida in relation to thirteen habitat variables – 8 physical and chemical water parameters and 5 selected habitat features – was analyzed using multivariate ordination analyses. Due to a long gradient, a canonical correspondence analysis (CCA) was used (Oksanen 2013). It was calculated on the basis of a matrix with any data transformations (O'Hara, Kotze 2010). The Monte Carlo permutation test was used to check whether the leech species were statistically significantly correlated with the measured habitat variables. Multivariate statistics were carried out using CANOCO 4.5 for Windows (ter Braak, Smilauer 2002).

Non-measurable environmental parameters used in ANOVA and CCA analyses were encoded as follows: pollution with sewage from the plant in Kluczkowice: 0 – above the source of pollution (pure water), 1 – far below the source of pollution (purified water, slightly polluted), 2 – close to the source of pollution (water strongly polluted); the degree of development of littoral and submerged vegetation: 0 – very poor, 1 – poorly developed, 2 – well-developed; surroundings: 0 – village, 1 – meadow, 2 – forest.

### Results

Out of the nine collected species, *Erpobdella vilnensis*, *E. octoculata* and *Helobdella stagnalis* were most abundant and accounted for 40.4%, 30.9% and 14.9% (respectively) of the total number of collected

leeches – Table 2. *Erpobdella octoculata* and *E. vilnensis* were recorded in the largest number of sites. *Erpobdella nigricollis* was collected only from the ponds, and *Haemopsis sanguisuga* only in the stream below the source of pollution. No leeches were found in the wellspring or its outflow (Localities 1 and 2). In the other sites, the occurrence of individuals of two to six leech species was observed – Table 2.

**Table 2**

Contribution of particular leech species [%] in the abundance of the Hirudinida assemblage in the examined sites. Numeration of the sites as in the text and in Fig. 1.

| Species                        | Locality number |   |    |    |    |    |    |
|--------------------------------|-----------------|---|----|----|----|----|----|
|                                | 1               | 2 | 3  | 4  | 5  | 6  | 7  |
| <i>Erpobdella nigricollis</i>  | 0               | 0 | 0  | 9  | 0  | 0  | 0  |
| <i>E. octoculata</i>           | 0               | 0 | 0  | 23 | 80 | 16 | 71 |
| <i>E. vilnensis</i>            | 0               | 0 | 85 | 0  | 0  | 74 | 28 |
| <i>Glossiphonia complanata</i> | 0               | 0 | 4  | 0  | 5  | 5  | 0  |
| <i>G. concolor</i>             | 0               | 0 | 0  | 4  | 0  | 5  | 0  |
| <i>G. nebulosa</i>             | 0               | 0 | 11 | 4  | 0  | 0  | 0  |
| <i>Haemopsis sanguisuga</i>    | 0               | 0 | 0  | 0  | 5  | 0  | 0  |
| <i>Helobdella stagnalis</i>    | 0               | 0 | 0  | 56 | 10 | 0  | 0  |
| <i>Hemiclepsis marginata</i>   | 0               | 0 | 0  | 4  | 0  | 0  | 0  |

Statistically significant correlations with the occurrence of leeches were found for four of the analyzed physical and chemical factors. The following values increased with the distance from the source: the abundance of *E. octoculata* ( $r_s=0.52$ ), the total number of leeches ( $r_s=0.42$ ) and the species richness of leeches ( $r_s=0.47$ ). The following values were positively correlated with water temperature: the abundance of *E. octoculata* ( $r_s=0.50$ ) and *H. stagnalis* ( $r_s=0.42$ ), the total number of leeches ( $r_s=0.42$ ) and the species richness ( $r_s=0.42$ ). The following values increased with a water depth: the abundance of *E. nigricollis* ( $r_s=0.38$ ) and *H. stagnalis* ( $r_s=0.50$ ), the total number of leeches ( $r_s=0.37$ ) and the species richness ( $r_s=0.36$ ). On the other hand, the number of *H. stagnalis* was negatively correlated with the current velocity ( $r_s=-0.40$ ).

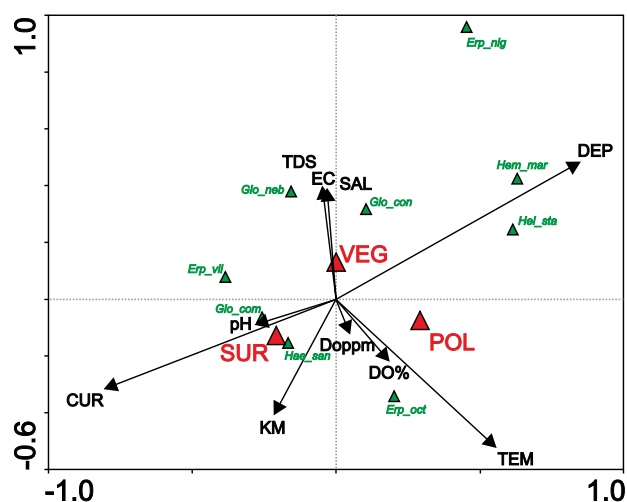
*Erpobdella octoculata* occurred most often in the polluted waters, less frequently in the partially self-purified waters and was absent in clean waters: H (2, N = 35) = 13.01974, p = 0.0015. *Haemopsis sanguisuga*

occurred only in waters affected by pollution:  $H(2, N = 35) = 10.91743$ ,  $p = 0.0043$ . The highest species richness of leeches was recorded in the polluted waters, followed by lower species richness in partially self-purified waters, and low species richness in clean waters:  $H(2, N = 35) = 6.114156$ ,  $p = 0.0470$ . The type of catchment was correlated with the abundance of *E. vilnensis* ( $H(2, N = 35) = 9.916166$ ,  $p = 0.0070$ ), the total number of leeches ( $H(2, N = 35) = 10.34576$ ,  $p = 0.0057$ ) and species richness ( $H(2, N = 35) = 9.520443$ ,  $p = 0.0086$ ). The meadow type of catchment was most favorable, followed by the forest type of catchment.

The level of development of littoral and submerged vegetation was correlated with the abundance of *Glossiphonia nebulosa* ( $H(2, N = 35) = 7.954545$ ,  $p = 0.0187$ ), the total number of leeches ( $H(2, N = 35) = 9.901784$ ,  $p = 0.0071$ ) and species richness ( $H(2, N = 35) = 9.257297$ ,  $p = 0.0098$ ). A very clear preference towards the waters with well-developed vegetation was noted in all instances.

The CCA analysis in Fig. 2 contains data on the habitat associations of individual species of leeches and shows the effects of environmental variables on the Hirudinida composition as a whole. The matrix used in the CCA analysis included all of the species for every stenotopic taxon, occurring even in small numbers, is essential in the analysis of longitudinal distribution in relation to environmental variables. The Monte Carlo permutation test was performed to show significance of the first canonical axis as well as all canonical axes together. The test of the first canonical axis was significant at  $p = 0.006$  ( $F = 3.91$ ), while the values for all four axes at  $p = 0.024$  and  $F = 1.63$ , respectively. Eigenvalues are measures of their importance: the first eigenvalue is 0.72, the second is 0.40. The sum of all eigenvalues is 2.55 and the variables used in the ordination explain 62% of the total variability in Hirudinida. Species-environment correlations for two first axes were 0.95 and 0.85.

Analysis of the CCA biplot shows that depth, current, temperature and vegetation are the most important factors for the distribution of leeches in the examined stream. Water pollution POL ( $r = 0.87$ ) and water depth DEP ( $r = 0.81$ ) are strongly correlated with the first CCA axis; the correlation with the water current CUR is slightly weaker ( $r = -0.76$ ). *Hemiclepsis marginata* and *H. stagnalis* clearly prefer the deeper parts of the stream, while



**Figure 2**

The CCA biplot of Hirudinida species in relation to 13 environmental variables. Abbreviations: Erp\_nig – *Erpobdella nigricollis*, Erp\_oct – *E. octoculata*, Erp\_vil – *E. vilnensis*, Glo\_com – *Glossiphonia complanata*, Glo\_con – *G. concolor*, Glo\_neb – *G. nebulosa*, Hae\_san – *Haemopsis sanguisuga*, Hel\_sta – *Helobdella stagnalis*, Hem\_mar – *Hemiclepsis marginata*, TEM – temperature, pH – potential of hydrogen, DO% – dissolved oxygen [%], DOppm – dissolved oxygen [ $\text{mg dm}^{-3}$ ], EC – electrical conductivity, TDS – total dissolved solids, SAL – salinity, DEP – depth, CUR – current velocity, VEG – stage of vegetation development, POL – pollution, SUR – surroundings. Nominal variables are marked in green.

*Glossiphonia complanata* and *H. sanguisuga* prefer shallower places with a moderate current situated in forests. At the same time, *G. complanata* shows a strong association with the sites with the highest pH. The second axis is correlated with vegetation ( $r = 0.70$ ). *Glossiphonia nebulosa* and *Glossiphonia concolor* show clear preferences for high values of EC, SAL and TDS. The occurrence of *Erpobdella octoculata* is limited to the most polluted sites, while *E. vilnensis* and *G. nebulosa* are associated with clean waters.

## Discussion

Many European leech species commonly occur in both lotic and lentic habitats (Sawyer 1974), but some of them show preference for one habitat or the other (Kubova et al. 2013). In the Wrzelowianka Stream, there are suitable mesohabitats and conditions

characteristic of both habitats (the main stream and ponds). Therefore, we have recorded taxa preferring different habitats. We recorded the presence of *E. vilnensis* and *G. complanata* exclusively in the stream, which, according to Kubova et al. (2013), prefer lotic ecosystems. *Helobdella stagnalis* occurred abundantly and *E. nigricollis* occurred only in fish ponds. *Erpobdella nigricollis* is a typical species of lakes, especially small reservoirs where vascular vegetation is well developed (Lukin 1976; Agapow, Bielecki 1992). The leech often occurs in river sections with slow or no flow (Bening 1924) and this pattern of distribution was observed in our study. In fish ponds, high abundance and species diversity was observed. This section and the upper part of the stream were surrounded by meadows and were characterized by abundant littoral and aquatic vegetation. This corresponds to the pattern described in the literature on macrofauna in general (Moss, Timms 1989; Żbikowski et al. 2010; Spyra 2011) and leeches in particular (Kubova et al. 2013). Among others, this can be caused by the insolation level; a higher density of macroinvertebrates can be observed in insolated habitats as compared to overshadowed areas (Spyra 2011). The food availability determined the distribution and abundance of predators and parasites, including leeches. While shading caused by riparian vegetation limited the number of benthofauna, the presence of aquatic vegetation affects its increase. Although an increase in shading caused by the presence of floating leaves of nymphaeids is unfavorable to periphyton and epiphytic fauna (McAbendroth et al. 2005), the heterogeneous system of rhizomes and roots is very favorable to benthic fauna (Cremona et al. 2008, Żbikowski et al. 2010). Kubova et al. (2013) found that the diversity of benthos together with the percentage cover of canopy are the best predictors of leech species composition.

Changes in the dominance among Hirudinida caused by insolation were also demonstrated: in overshadowed localities, representatives of the family Glossiphoniidae dominated, while in insolated localities – Erpobdellidae (Spyra 2011). Both leech families substituting for each other are characterized by negative phototaxis; the change in the abundance proportion must be caused by an additional environmental factor. Possibly, it is the food availability connected with the presence

of macrophytes, whose role in the creation of habitats is emphasized in many studies (e.g. Sawyer 1986, Bielecki et al. 2004, Kubova et al. 2013). Macrophytes have an impact on physicochemical properties of waters such as transparency and the content of biogenic compounds (Grzybowski 2014), and intensive photosynthesis processes may result in an increased pH value (Neurminen 2003, Harrison et al. 2005). However, the most important factor resulting from the presence of aquatic vegetation seems to be an increase in the food availability.

The distribution of *H. sanguisuga* (Timm et al. 2011) – insensitive to hydromorphological conditions in the Wrzelowiecki Stream is probably also determined mainly by the abundance of food and the feeding behavior. The former leads an amphibious lifestyle and is usually found on the border between water and land (Koperski 2003) where it searches for prey, which may explain the association of this species with a shallow water zone.

The occurrence of *H. marginata* in only one site confirms the preference of this species for lentic habitats reported by Kubova et al. (2013). But we should also emphasize the importance of the feeding behavior. Amphibians and fish are very important elements in the life cycle of the leech (Pawłowski 1968). Within the examined localities, it occurred mainly or almost exclusively in the ponds.

With regard to the dominant species, physical and chemical conditions seem to be the most important factors for their distribution. *Erpobdella vilnensis* is a typical species in upland and mountain areas (Bielecki et al. 2011, Utevsky et al. 2012). Normally, the leech inhabits streams with minor anthropopressure, hence its presence can be attributed to the high quality of the aquatic environment. In our study, the leech was not found in the stream sections affected by human impact, but we should emphasize that these localities are also characterized by a considerably reduced flow rate.

As opposed to *E. vilnensis*, *E. octoculata* has been described as a negative indicator of the watercourse quality (Koperski 2005). It occurs in large numbers in the mesosaprobic zone of running waters (Kazanci et al. 2014) and its contribution in the assemblage of Hirudinida is correlated with the degree of pollution (Koperski 2005). In the examined stream, the occurrence of *E. octoculata*

was noted in fish ponds and below, where the intensity of anthropogenic impact increased. Thus, our observations have confirmed the relation of *E. octoculata* with polluted waters, and we believe that the physiological resistance, to a certain degree, to water pollution has allowed the leech to spread throughout the Palearctic.

However, it should be stressed that although *E. vilnensis* and *E. octoculata* are characterized by different responses to the degree of pollution, both of them were present in our study, at localities with clear anthropopressure. Depending on the environmental conditions, changes were observed in the quantitative proportions of leeches representing different species. The coexistence of these two species was described by many authors (e.g. Schenkova et al. 2007; Beracko, Rogńska 2014).

Therefore, another factor had the main impact on the distribution of the two species. Results of our study demonstrated that it may be the water temperature. This factor has a significant effect on maturation (Elliott 1973a) and feeding activity of leeches (Elliott 1973b).

Our results have also confirmed the previously observed preferences of *H. stagnalis* for waters with a high trophic status and low flow (Mann 1955). They differ from Koperski's (2005) observations that *H. stagnalis* is a characteristic species in waters of high quality. However, individuals of the species also occur in clean headwaters as reported in the literature (Koperski 2005; Grosser, Pešić 2006), and data concerning the waters with a significant content of organic matter are related to stagnant waters. In our study, this taxon was represented abundantly in the polytrophic carp-ponds. Perhaps these leeches, having a choice of different types of habitat, as in the case of the examined stream, prefer places with a low flow and high trophic status. If they inhabit running waters, they are associated with unpolluted sections. Perhaps the different habitat preferences of this species in lotic and lentic ecosystems could be considered, an approach coherent with Koperski's (2005) observation about the indicative role of *H. stagnalis* as a species specific to water courses of high quality. Many researchers (e.g. Sandin 2003, Timm et al. 2011) have emphasized the significance of flow velocity and parameters correlated with velocity in the distribution of benthofauna. In respect to *H. stagnalis*, the water flow velocity which strongly

affects oxygenic and food rates, possibly determines the species behavior. In highly-oxygenated waters, these animals burn more energy to find food, while in eutrophic waters generally abundant in food, they probably require smaller amounts of energy and less oxygen. Probably, specific physiological mechanisms facilitate both of these life strategies. In the case of *H. stagnalis*, Mann (1956) demonstrated the discrete changes in the rate of oxygen consumption in the conditions of limited availability of this element in water, and the experimental studies on *Nephelopsis obscura* (Davies, Thomas 1991) demonstrated a smaller impact on the prey occurring with the same density, along with a decrease in the degree of water oxygenation.

## Conclusion

In conclusion, the hypothesis of this study has been corroborated, thus the species composition of leech assemblages is shifting along with changes in the habitat conditions of a watercourse. The results of our study indicate that the hydromorphological conditions and food availability have a major significance for the distribution of leeches in the examined watercourse. The vegetation, both aquatic and growing in the catchment, should also be considered as a significant factor in terms of leech distribution. Especially the submerged vegetation can affect the local habitat conditions including the food resources.

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