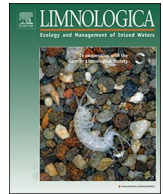




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## Factors shaping leech (Clitellata, Hirudinida) assemblages on artificial and natural substrata in urban water bodies

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### ABSTRACT

Leech assemblages on the bottom substrata and artificial plates deployed in various types of waterbodies located in an urbanized area (Olsztyn, Northeastern Poland) were examined to identify environmental factors responsible for their distribution. The leech assemblages were not affected by physicochemical parameters of water. Important factors shaping the taxonomic composition of leech assemblages were the type of a waterbody (lakes, rivers, ponds) and human impact (including the land use, substratum quality and shoreline type). Waterbody type and human impact affected species richness, with lower values observed in small ponds and under stronger anthropogenic impact. The comparison of assemblages sampled manually from the bottom and from artificial plates deployed in the environment revealed that the artificial plates were a good alternative for traditional methods of leech sampling, particularly at strongly anthropogenically modified locations. Furthermore, several rare species: *Alboglossiphonia striata*, *A. hyalina*, *Glossiphonia nebulosa*, *Hirudo medicinalis* and *Dina* sp. were found in the urbanised areas under study, pointing out to their potential role in the conservation of these taxa.

### 1. Introduction

Leeches are one of the most abundant and widely distributed group of benthic invertebrates (Koperski, 2003; Strzelec et al., 2010). They are an important component of aquatic ecosystems (Raczyńska et al., 2014), functioning as predators or parasitic canivores (Elliott and Dobson, 2015). They are also indicators of water chemistry and biodiversity and the presence of specific taxa of Hirudinida is associated with the ecological status of water bodies and occurrence of certain animals (Govedich et al., 2010). Therefore, the knowledge of environmental factors shaping leech assemblages is relatively good (Mann, 1962; Sawyer, 1986; Elliott and Dobson, 2015). According to Sawyer (1986) they include, in approximately decreasing order of significance: food availability, type of bottom substratum, water depth, currents (lentic or lotic waters), size and form of a water body, water hardness, pH, water temperature, minimum concentration of dissolved oxygen, siltation, turbidity and salinity.

Leeches are usually associated with solid submerged objects and hard surfaces (Agapow, 1980; Elliott and Dobson, 2015), which are often introduced to the aquatic environment by human activity, either as artificial structures used for exploitation of the water resources or

solid waste discarded to water (Czerniawska-Kusza, 2004; Czarnecka et al., 2009). That is why they can be expected to occur in urban, anthropogenically modified locations. Nowadays, urbanized areas develop at a very fast rate. They quickly increase in size and start to play more and more important role in the landscape quality (McIntyre et al., 2000; Marzluff, 2005). The loss of natural habitats is regarded as the most important cause of species extinctions. On the other hand, some species thrive in anthropogenic habitats, finding very suitable living conditions in such locations. These also include some rare and/or endangered taxa (Buczyński et al., 2008; Bielecki et al., 2011; Lewin et al., 2015), which may indicate the role of these habitats in biodiversity protection. Therefore, in the light of these facts, the importance of studies on the biodiversity and species composition in aquatic habitats in urbanized areas seems high and leeches, as organisms commonly associated with solid surfaces, are very suitable subjects for such investigations.

Nevertheless, the number of studies dealing with leech assemblages in urban locations (Koperski, 2010b; Bielecki et al., 2011) is relatively low. Perhaps, the difficulties in sample collection might have caused this scarcity. Usually habitats in urbanised areas are strongly modified by human activity and contain large concrete surfaces, fascine

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embankments and high amount of anthropogenic waste, which make the use of standard sampling methods difficult. Commonly applied methods of leech collecting suffer from a number of limitations, potentially affecting the results (Koperski, 2003; Adamiak-Brud et al., 2015). One of the most common sampling methods is manual collection in a specified time unit (Saglam et al., 2008; Darabi-Darestani and Malek, 2011; Kubová et al., 2013). Although such sampling should be conducted with the same effectiveness at each site, the final result depends on many factors, including the activity and experience of a collector (Meier et al., 1979). Moreover, leeches have strongly clumped distribution and occupy mainly solid surfaces, such as stones, macrophytes and anthropogenic objects (Sawyer, 1986; Elliott and Dobson, 2015). This precludes objective selection of sampled surfaces and comparison of assemblages occupying different bottom types (Young and Ironmonger, 1981). On the other hand, the use of a benthic dredge or other conventional benthic samplers fails on the hard, stony bottom (Boothroyd and Dickie, 1989; Koperski, 2003) as well as in anthropogenically modified locations and can artificially downgrade the diversity of leeches in the environment, given the fact that they avoid soft sediments (Agapow, 1980; Elliott and Dobson, 2015).

Therefore, the use of artificial plates seems to be an interesting alternative for collecting faunistic material for comparative studies of leeches, which commonly exhibit a strong thigmotaxis and preferences for anthropogenic objects (Czerniawska-Kusza, 2004; Czarnecka et al., 2009). An important advantage of this method is the elimination of the impact of physical differences between the substratum types at studied sites, as well as the possibility of comparisons between different habitats and water body types. Moreover, artificial plates are especially recommended for aquatic invertebrate sampling at sites where other methods may be difficult to apply (Rosenberg and Resh, 1982; De Pauw et al., 1986), including anthropogenically affected locations.

The purpose of our study was to determine (1) the taxonomic composition of leeches occupying bottom substrata and artificial plates in different types of water bodies from an urbanized area, (2) environmental factors affecting their occurrence and distribution in these water bodies and (3) efficiency of leech collection using artificial plates in urbanised areas. We hypothesized that leech species richness and assemblage composition would be affected by sampling type (manual collection from the bottom vs. artificial plates) and the strength of local human impact.

## 2. Study site

Leeches were collected between August and October 2013 at 24 sites located in 13 freshwater water bodies placed within the city of Olsztyn and its surroundings (Mazurian Lake District, northeastern Poland). Olsztyn is the largest city in north-eastern Poland with regard to the area (88 km<sup>2</sup>) and population (173 831) (according to the data of the Statistical Office, Olsztyn 2014). Many aquatic ecosystems are located within the city borders, including a large number of small water bodies and lakes, usually intensely utilized for tourism and recreation. For the study, six lakes, two rivers and five small ponds were selected. Twelve sampling sites were placed in lakes: Lake Długie (S1-S2), Lake Kortowskie (S3-S4), Lake Redykajny (S5), Lake Skanda (S6-S7), Lake Tyrsko (S8) and Lake Ukiel (S9-S12). Seven further sites were located in two lowland rivers: Lyna River (S13-S17) and Wadąg River (S18-S19). Finally, five sites were situated in small ponds (S20-S24) (Fig. 1). The sampling sites differed from one another in terms of human impact, showing small or large degree of anthropogenic transformation. The characteristics of the studied water bodies is given in Tables 1 and 2. The data on the type of land use in the vicinity of each site, their surface area (ha), width (m) and detailed localities have been determined on the basis of satellite and hybrid maps from [www.geoportal.gov.pl](http://www.geoportal.gov.pl). The information on the bottom types, water level variability, types of shoreline and additional factors, including the presence of multi-apartment buildings, trash, chemical pollution, intensive angling, dredging

the bottom, removing of macrophytes is based on the authors' own observations.

## 3. Materials and methods

Three artificial substrates (plates) were placed at each sampling site in the near shore area of the selected water bodies, between July and October 2013. Artificial substrates used in the study were deployed horizontally in one row, at a depth of 30 cm, directly on the bottom, about 5 cm apart. Transparent, corrugated polyethylene 40 × 40 × 0.1 cm plates were used on the basis of the previous substratum preference studies (Adamiak-Brud et al., 2015). Leeches were collected from plates after their 4 week exposition in the environment in three repetitions (two in summer: 8–14 August and 3–6 September and one in autumn 1–4 October). As leeches are known to prefer the bottom surfaces of submerged objects (Adamiak-Brud et al., 2016), at each sampling site leeches were collected manually from the downward surfaces of artificial plates. After collection, the plates were cleaned of debris and organisms with a brush and placed again in the environment. Moreover, on 8–14 August leeches were collected manually from the bottom in the vicinity of the plates during 2 h. The following water parameters were measured in August and October (except sites 3, 5, 6, 8, 10, 21, 24, see Tables 1–2): concentrations of dissolved oxygen, ammonium ions, nitrates, chlorides, sulphates, phosphates and calcium as well as conductivity and pH. Leeches collected from the plates were transferred to the laboratory, where they were relaxed in 10% ethyl alcohol (10 min) and preserved in 70% ethyl alcohol. The collected leeches were classified to the species level according to Sawyer (1986), Bielecki (1997) and Elliott and Dobson (2015).

### 3.1. Statistical analysis

To reveal relationships between species and environmental variables, we conducted a series of canonical correspondence analyses (CCA) with forward variable selection, determining the most important factors related to the composition of leech assemblages. For the preliminary analysis, we used a dataset containing all environmental factors (Table 3), excluding only those which were strongly inter-correlated with the others. As some variables (chemical parameters and the presence of waste) were not checked at all dates and sites, we used a subset of samples with all the variables measured for this analysis. It demonstrated the lack of relationship between chemical parameters and leech species distribution (see the Results), which allowed us to conduct another CCA using all the August samples, where both sampling methods were used and only those environmental variables, which were always determined (Table 3). As the abundances on the plates and in the samples collected manually from the bottom were not comparable, we used percentage shares of species in the aforementioned analyses. Finally, we conducted a third CCA using the log-transformed abundances of leech species on the artificial plates only. A similar analysis using only the data from the manual sampling was not possible due to too small number of samples. The significance of the relationships between leech species and environmental variables along the particular CCA axes was tested using permutation tests with 500 replicates. Rare species (represented by less than 10 individuals or occurring in less than 5 samples) were removed from the dataset to reduce noise. One sample with no leech individuals (site 20 in October) was excluded from the CCA analyses. Moreover, as on some occasions the plates were damaged before processing or the sampling was not possible (site 1 in September and October, site 10 and 24 in October and all the samples from site 13), the final numbers of samples in the above-mentioned analyses were: 46, 46 and 64, respectively. The CCA analyses were carried out using Vegan 2.4.0 package for R (Oksanen et al., 2016).

We tested the differences in taxonomic compositions of leech assemblages using a PERMANOVA (with Bray-Curtis distances) with the

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