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# The use of bdelloids in reference to rotifer biocoenotic indices as an indicator of the ecological state of small field water bodies: The effect of macrophytes, shading and trophic state of water

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### ABSTRACT

Species identification of many rotifers is very difficult due to e.g. body contraction in the preserved material. In the case of bdelloids, it is even more problematic as they can only be determined when alive. Therefore, a necessity for developing a method of using the class Bdelloidea for environmental analyses arose, so I examined them as a whole group. This is an approach that reduces extensive, time-consuming and particularly difficult taxonomic identification of certain species. The occurrence of bdelloids against various rotifer community indices in a mosaic of habitats of 220 small water bodies in an agricultural landscape was subjected to both abiotic and biotic factors so as to measure the extent of their reaction and the possibility of their usage in the assessment of environmental conditions. Despite their preference for forest ponds, bdelloids were also a very common and abundant element of field aquatic ecosystems. Due to their specific sensitivity bdelloids serve as a valuable ecological indicator which can be used for environmental assessment of habitat quality in small water bodies in the agricultural landscape and also for monitoring appropriate conservation actions for ponds. Bdelloids are typically macrophyte-associated organisms, selectively choosing Phragmites stands, a transitional area between aquatic and terrestrial ecosystems. Their response to the environment was similar to the littoral fraction and rotifer diversity indices, which were found to be in opposition to the eutrophic community associated with the open water area in large-surfaced fish ponds. Moreover, the increase in bdelloids in ponds of low human impact supports their potential for the diagnosis of natural ponds of low transformation level. Their preference towards ponds with an increasing layer of nymphaeids and pleustophytes also supports their role in diagnosing goodquality waters as floating-leaved macrophytes are known to improve water quality. It is recommended that bdelloids as a group can be used for the determination of the level of heterogeneity (connected with macrophyte occurrence) and the level of shade (generated by macrophyte cover) as well as the water quality state of small water bodies.

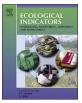
#### 1. Introduction

While the functioning of monogonont rotifer (Cl. Monogononta) assemblages has received much attention from scientists over recent decades (Segers, 2008), knowledge of the biology and ecology of bdelloids (Cl. Bdelloidea) is still unsatisfactory. Their poor recognition is due to several reasons. One of them is habitat specificity, connected with their living in places that are generally rarely examined. Bdelloids inhabit not only a range of freshwater environments such as bogs, leaf-litters, meadows, moist soils but also mosses or tree holes (Pejler and Bērziņš, 1993; Ricci and Caprioli, 2005; Kuczyńska-Kippen, 2008; Devetter, 2009; García et al., 2017). They are also known to live abundantly among the epizoon of sponges (Bołtruszko and Ejsmont-Karabin, 2013) or within psammic communities (Lokko and Virro,

2014; Lokko et al., 2017). However, apart from 'extraordinary' habitats, such as uranium mine pit water bodies (Ferrari et al., 2015) or extremely cold habitats (Iakovenko et al., 2015), they may also be found in other rather typical aquatic ecosystems, such as lakes, streams and springs (D'Ambrosio et al., 2016) as well as in small water bodies, which belong to aquatic environments that often undergo severe changes in abiotic features (Basińska et al., 2010), especially pronounced changes in water level which can significantly affect the overall functioning of the ecosystem (Gołdyn et al., 2015). Such conditions can in some cases favour bdelloids, which are known to be able to withstand extremes in their surrounding environment. Specific features, such as morphological structures, obligate parthenogenesis as well as anhydrobiosis (Ricci, 1987), responsible for their great adaptability, allow this group of rotifers to inhabit such a great variety of

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habitats and thus we can expect to find them in semiaquatic areas or harsh environments with sharp fluctuations of abiotic conditions, such as the examined ponds.

Another cause for the poor recognition of bdelloids is connected with a range of problems referring to taxonomic identification. Certain species remain unrecognisable due to the high level of specimen contraction in the process of sample preservation (Koste and Shiel 1986; Garraffoni et al., 2017), so live material is advisable for studies of these rotifers or some specific methods for their preservation. For bdelloids that have no lorica and thin body structures, anesthetization under low temperature is necessary in order to fix the specimens without contraction (Garraffoni et al., 2017). However, it is usually the case that only preserved samples are at scientists' disposal. This is why we decided to analyse bdelloids as a whole group so as to find out whether there are any particular patterns in environmental characteristics that will force them to build abundant communities in the case of small water bodies.

Even though bdelloids are such a difficult group to work with, they undergo a wide range of scientific interest, especially connected with their unique features, among which are, for example, their degenerate tetraploidy, horizontal gene transfer, starvation, high adaptability to live in unstable habitats manifested through anhydrobiosis, resistance to desiccation, strictly parthenogenetic reproduction or selective feeding (Welch and Meselson, 2000; Ricci and Fontaneto, 2009; Hespeels et al., 2014; Ricci, 2017). While bdelloid evolutionary phenomena have been frequently investigated, their ecological role is far less recognised, apart from their affinity for extreme environments. Thus, information that would provide more details on the ecological role of bdelloids along with their habitat preferences is greatly needed.

As part of the rotifer community bdelloids are poorly recognised compared to monogonont rotifers, and their indicative role is even less realised. Therefore, a need for the identification of triggers responsible for bdelloid distribution arises. It is not only basic abiotic features that can play a role in determining their occurrence. The type of habitat, even in the case of small surface water bodies, may also be important. A division of the pond's area into various habitats may create different conditions and thus preference of bdelloids toward a specific habitat can also be expected, especially as these rotifers exhibit adaptations to a variety of severe environments. This is why they may dominate over other rotifers in non-advantageous habitats. Therefore, the main aim of the study was to define the most important parameters that affect the prevalence of bdelloids over monogononts with special emphasis on features connected with habitat structure in reference to the level of complexity. Moreover, a thick layer of macrophytes on the surface of ponds may reduce sediment resuspension but at the same time cause oxygen depletion and so affect the fish kill (e.g. Barko and James, 1998). Thus, it was hypothesised that such harsh conditions will favour bdelloid occurrence in small water bodies.

Another important aspect concerning small water bodies is connected with the assessment of trophic conditions. As in the case of monogononts their role as indicators of mesotrophic or eutrophic waters is widely known (Ejsmont-Karabin, 2012; Špoljar, 2013), yet bdelloids have never been associated with the trophic state of water bodies. This is why the gap concerning bdelloids in reference to water trophy should also be filled.

Bdelloids are described as resistant to adverse factors and such conditions are often attributed to small and shallow water bodies which undergo great variation in the water regime throughout the year. This is why I decided to examine bdelloids against the background of other rotifer community indices in terms of their usefulness in the identification of the most important determinants of their occurrence. This attempt provided a basis on which to analyse the indicative role of this group of rotifers inhabiting small water bodies within the agricultural landscape. It is not only frequent phases of water shortage that are a typical phenomenon here. Ponds located within fields or rural areas are prone to a number of adverse conditions such as mechanical damage of buffer strips leading to degraded buffer zones and littoral vegetation belts, overfertilisation, backfilling, water pollution or littering (Juszczak and Kędziora, 2003; Kristensen and Globevnik, 2014). Moreover, the effects on rotifers of shading caused by macrophyte cover and a surrounding tree band are also poorly known. However, it would be extremely valuable for rotifer indicative strength to identify their response, reflecting their tolerance and persistence, to shade. Most of these threats refer to ponds located within the agricultural landscape such as the examined group of small water bodies.

Ecological analyses are usually based on the occurrence of species, which reflects their responses to environmental factors. As each species may react in a different way, a taxonomic approach may cause difficulties in assessing the real impact of ecological characteristics. Thus, an ecological approach often seems to be more suitable than taxonbased analyses. Therefore, bdelloids were examined as a whole group in reference to various functional groups of rotifers. The specific aims of this study included answering the following questions: how often do bdelloids occur in small water bodies; which habitats do they preferably inhabit in ponds; what are the main triggers of their occurrence in ponds; is their response to environmental parameters similar to any other biocoenotic features of the rotifer community? My final attempt will concern whether bdelloids as a group can serve as a valid instrument in ecological analysis.

#### 2. Materials and methods

In total, 220 small water bodies were studied in the years 2004-2013 in order to find the best predictors of bdelloid occurrence in a mosaic of habitats of small freshwater ecosystems. The study was conducted during the optimum summer season (late July-August) in accordance with recommendations of Kuczyńska-Kippen (2001) and Eismont-Karabin (2012) who demonstrated that summer zooplankton assemblages are in the optimum and remain under the impact of trophic state factors. Thus, this season should be chosen for comparative analyses. In order to reduce the diurnal variation in the abiotic features all samples were taken at the same time - around midday. All small water bodies included in this study differed in respect to their origin, trophic conditions, morphometry and level of human impact. They were located within the Wielkopolska Province (area: ~30,000 km<sup>2</sup>), centralwestern Poland. The main feature of the economy in this region is highproductive agriculture, as the major part of the Province (almost 60%) is typically agricultural, including both arable land as well as set aside land, pastures and meadows.

In order to avoid the effect of the type of catchment area on zooplankton (Kuczyńska-Kippen and Joniak, 2010) all ponds were situated in an agricultural/rural catchment area of central-western Poland.

In all, 445 sites were taken into consideration with 106 sites located within elodeids (with dominating sites among: *Ceratophyllum demersum*; *Ceratophyllum submersum*, charoids; *Myriophyllum* spp.), 65 within helophytes (with dominating sites among: *Phragmites australis; Typha* spp.). The majority of examined sites (274) were covered by the open water area, which reflects the % participation of particular zones in freshwater. All biometric measurements of plants for elodeids and helophytes are described in detail in Nagengast and Kuczyńska-Kippen (2015).

Rotifer samples were taken from each site in triplicate, using a calibrated vessel in the open water area and a plexiglass core sampler (Ø 50 mm; length 1.5 m) in vegetated stations. The samples were concentrated by a 45  $\mu$ m plankton net and fixed immediately with 4% formalin. Samples for taxonomical and quantitative analyses were sedimented in the laboratory and were finally thickened into a volume of 10 ml. Species composition and rotifer abundance were determined with a light microscope. Other details concerning sampling and counting methods were described in previous papers by Ejsmont-Karabin and Kuczyńska-Kippen (2001) and Kuczyńska-Kippen and Joniak (2016).

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