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What is the optimum sample size for the study of peatland testate amoeba assemblages?

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Abstract

Testate amoebae are widely used in ecological and palaeoecological studies of peatlands, particularly as indicators of surface wetness. To ensure data are robust and comparable it is important to consider methodological factors which may affect results. One significant question which has not been directly addressed in previous studies is how sample size (expressed here as number of *Sphagnum* stems) affects data quality. In three contrasting locations in a Russian peatland we extracted samples of differing size, analysed testate amoebae and calculated a number of widely-used indices: species richness, Simpson diversity, compositional dissimilarity from the largest sample and transfer function predictions of water table depth. We found that there was a trend for larger samples to contain more species across the range of commonly-used sample sizes in ecological studies. Smaller samples sometimes failed to produce counts of testate amoebae often considered minimally adequate. It seems likely that analyses based on samples of different sizes may not produce consistent data. Decisions about sample size need to reflect trade-offs between logistics, data quality, spatial resolution and the disturbance involved in sample extraction. For most common ecological applications we suggest that samples of more than eight *Sphagnum* stems are likely to be desirable.

Keywords: Bioindication; Protist; Sample Size; Testate amoebae; Transfer function; Wetland

Introduction

Testate amoebae are a polyphyletic group of protists defined by the presence of a test (shell) (Meisterfeld 2002). Testate amoebae are abundant in a wide variety of habitats but are particularly abundant in freshwater wetlands where they can be the dominant group of heterotrophic protists (Gilbert et al. 1998; Mitchell et al. 2008). Over recent years there has been considerable interest in the application of testate amoebae as bioindicators for a wide variety of environmental changes (Payne 2013). The most widespread of these uses has been as indicators of water table depth in palaeoecological studies from peatlands (Charman 1999; Qin et al. 2013; Van Bellen et al. 2014). After numerous studies over the last 25 years it is now well-established that testate amoebae taxa have differing preferences for peatland surface wetness (usually expressed as water table depth). Transfer functions which attempt to quantify these optima in surface samples

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have been widely used to produce quantitative reconstructions of changing water table depth from peat cores (Payne et al. 2016).

As testate amoebae have become more widely studied in peatlands there has been an increasing focus on the testing and refinement of methods and interpretation. Studies have focussed on questions such as optimum preparation methods (Avel and Pensa 2013; Hendon and Charman 1997), sampling depth (Roe et al. 2017), taxonomic approach (Mitchell et al. 2014; Payne et al. 2011) and sample storage (Mazei et al. 2015). There are particularly important questions regarding the scaling relationships between sampling effort and data quality. Several studies have looked at the relationship between the number of individual tests counted under the microscope and the species richness (Mitchell et al. 2000; Warner 1990; Woodland et al. 1998) and composition (Payne and Mitchell 2009) of the assemblage identified. The influence of the size of sample analysed has been little considered despite extensive consideration in other contexts (Azovsky 2000; Heck et al. 1975).

Testate amoeba assemblages are known to show fine-scale spatial variation even in areas of relatively homogeneous vegetation and physical environment. In the most intensive study of this topic Mitchell et al. (2000) studied the testate amoeba assemblages of a *Sphagnum magellanicum* lawn in a Swiss peatland. Across a macroscopically homogeneous plot of only 40 × 60 cm these authors showed considerable variability in testate amoeba assemblages with clear spatial structuring of the species composition and large variability in biomass. Some individual taxa differed in relative abundance by an order of magnitude between adjacent samples. Another study of testate amoeba distribution in a macroscopically homogeneous *Sphagnum angustifolium* lawn has shown species-dependent spatial organisation down to a scale of 1 cm (Mazei and Tsyganov 2007).

Assuming this level of fine-scale spatial variability is typical for peatlands this raises the question: what is the optimum sample size for the determination of testate amoeba assemblages in ecological studies? The sample size considered in previous studies varies considerably from a single *Sphagnum* stem up to samples of more than 25 cm^2 which may represent dozens of individual stems (Jassey et al. 2012; Mitchell et al. 2000; Payne et al. 2006). It seems plausible that different sample sizes may lead to datasets which differ in important respects. In this study we analysed surface samples spanning the range of commonly used sizes in order to assess whether and how such differences affect data quality and to make recommendations for future studies.

Material and Methods

Study site and sampling

Samples for the study were collected in a mesotrophic peatland (53.125511°N, 45.841298°E) located in the forest-steppe zone of the East European Plain (Penza Region, Russia) in July, 2007 (Supplementary Fig. 1). The study area has a continental climate characterized by mean January temperature of −12 °C and mean July temperatures of +20 °C. Mean annual precipitation is 500 mm yr^{-1}, at the lower end of the range typical for northern peatlands (World Water and Climate Atlas, 1961–1991; New et al., 2002). The vegetation of the peatland is dominated by *Carex* spp. and *Sphagnum* spp.

To consider how sample size-assemblage relationships may differ between microhabitats we conducted sampling in three locations spanning the range of surface wetness and vegetation commonly encountered in northern peatlands. Biotope 1 was the driest with vegetation cover of *Sphagnum angustifolium* and *Polytrichum strictum* and a canopy of *Betula* sp., the measured water table depth was 26 cm. Biotope 2 was intermediate in wetness with open lawn vegetation of *Sphagnum palustre* and *Sphagnum magellanicum* and no trees, water table depth was 12 cm. Biotope 3 was a hollow with *Sphagnum squarrosum* and was the wettest of the sampling locations with a water table depth of 0 cm. In each location samples of different size (1, 3 and 8 *Sphagnum* stems) were extracted from the same location in three replicates and one larger sample of 16 stems was extracted giving a total of 30 samples. We focus on the number of *Sphagnum* stems as an index of sample size because this is easily determined in the field and frequently used by analysts. Sampled stems extended to a depth of 6 cm. Material sampled was *Sphagnum angustifolium* in Biotope 1, *Sphagnum palustre* in Biotope 2 and *Sphagnum squarrosum* in Biotope 3. This difference in *Sphagnum* species sampled was necessitated by the aim to consider a variety of assemblages. However it is important to note that this may influence results because different *Sphagnum* species may contain different test densities and may grow at different rates meaning that the same stem depth represents differing time periods. The samples were placed in plastic flasks and stored in 4% formalin to avoid the possibility of any post-sampling change in assemblage (Mazei et al., 2015).

Testate amoeba analysis

Samples were prepared for testate amoeba analysis following a water-based technique (Mazei and Chernyshov, 2011). Moss samples were suspended in deionised water and thoroughly shaken for 5 min. The suspension was carefully poured in to a Petri dish (10 cm diameter) and left to settle. Testate amoebae were identified and counted by direct microscopy with a dissecting light microscope (Biomed, Russia) at a magnification of 160×. Tests were identified based on Mazei and Tsyganov (2006). The full volume of each sample was counted and all tests recorded, live individuals were not differentiated.