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Testate amoeba analysis of lake sediments: impact of filter size and total count on estimates of density, species richness and assemblage structure

A. A. J. Wall · D. Gilbert · M. Magny · E. A. D. Mitchell

Abstract Testate amoebae are informative about palaeoecological conditions, but the methods generally used for their analyses in lake sediments differ from those used for their analyses in peats, making comparisons difficult. This study examines how filter mesh size and total number of individuals counted affect species richness, Shannon diversity, equitability, density and assemblage structure. We analysed the complete testate amoeba contents of six sediment samples from Lake Lautrey, France. The abundance of testate amoebae was high (1,403–10,870 shells cm^{-3}), and species smaller than 63 μm in both length and width represented up to 89% of total abundance and 43% of species richness. A simulation showed that using 47- or 63-μm mesh-size filters reduced inter-sample differences and changed the patterns of abundance, species richness and assemblage structure, causing loss of information and leading to potential erroneous palaeoecological interpretation. Rarefaction analyses suggest that although 170 shells are sufficient to assess the general structure of assemblages, such small sample sizes can underestimate species richness by overlooking taxa with relative abundances <4%. Total counts of 400 shells yield better estimates of assemblage structure and recover at least 50% of total species richness, although species with absolute frequencies below 2% may still be missed. Higher counts are required to obtain reliable estimates of species richness and assemblage structure in samples that have high testate amoeba densities but are dominated by a few small taxa. Further studies should determine the bioindicator value and functional roles of small and/or rare species in lakes and thus to what extent overlooking them affects palaeoecological interpretations.

Keywords Thecamoebians · Palaeoecology · Lake sediment · Diversity · Abundance · Filter mesh size

A. A. J. Wall (✉) · D. Gilbert · M. Magny
Laboratoire Chrono-Environnement, CNRS/UFC UMR 6249, UFR Sciences et Techniques, 16 route de Gray, 25030 Besançon Cedex, France
e-mail: adeline.wall@univ-fcomte.fr; adelinewall@yahoo.fr

D. Gilbert
e-mail: daniel.gilbert@univ-fcomte.fr

M. Magny
e-mail: michel.magny@univ-fcomte.fr

E. A. D. Mitchell
Ecosystem Boundaries Research Unit, Wetlands Research Group, WSL Swiss Federal Research Institute, Station 2, 1015 Lausanne, Switzerland
e-mail: edward.mitchell@wsl.ch

E. A. D. Mitchell
Laboratory of Ecological Systems, Ecole Polytechnique Fédérale de Lausanne, Station 2, 1015 Lausanne, Switzerland

E. A. D. Mitchell
Laboratory of Soil Biology, Institute of Biology, University of Neuchâtel, 2009 Neuchâtel, Switzerland

Introduction

Testate amoebae are ubiquitous protists that produce a characteristic shell called a test. The taxonomy of testate amoebae is largely based on the morphological characters of these shells (Meisterfeld 2002a, b). Shells are generally well preserved in lake sediments and peats (Mitchell et al. 2007) allowing the identification of testate amoeba fossils to the species level and inferences about past environmental conditions (Charman 2001). Testate amoebae are increasingly used as tools in palaeoenvironmental studies of lakes (Schönborn 1973, 1984; Ruzicka 1982; Tolonen 1986; Medioli and Scott 1988; Ellison 1995; Burbidge and Shröder-Adams 1998), ponds and peatlands (Warner 1990; Charman 2001; Gilbert and Mitchell 2006; Charman et al. 2007; Lamentowicz et al. 2008). Testate amoebae are also considered good indicators of land-use change (Reinhardt et al. 2005) and water and air pollution (Gilbert et al. 1998; Torigai et al. 2000; Nguyen-Viet et al. 2004, 2007, 2008; Mitchell et al. 2008). They feed on bacteria, algae, and fungi, and larger species prey on other protozoans and small metazoans (Mast and Root 1916; Heal 1963; Gilbert et al. 2000, 2003). Community structure may be influenced directly by changes in abiotic conditions and indirectly by variations in the structure of the microbial communities.

We believe two problems need to be addressed in order to improve the value of testate amoeba analysis as a reliable tool in palaeolimnological studies. The first problem is that methods generally used for testate amoeba analysis in lake sediments are different from those used for analysis in peats, making comparisons difficult (Warner 1990; Charman et al. 2000; Beyens and Meisterfeld 2001). The approach used in lakes normally excludes small species (<45 μm or even <63 μm) from the analysis while these smaller species are included in palaeoecological studies of peatlands. If small species represent an important part of the assemblage or have high bioindicator values, this might significantly reduce the usefulness of testate amoebae unless smaller mesh-size filters are used for sample preparation.

A second problem is that the total count size needed to obtain reliable estimates of assemblage structure, and especially the presence of rare species of high bioindicator value, has not been studied in detail in the context of palaeolimnology, although this question has been dealt with for peatlands (Payne and Mitchell 2009).

The objective of this study was to test an extraction method of testate amoeba shells from lacustrine sediments and compare it with currently used methods. We assessed the effect of using different filter sizes theoretically by using the morphometrical data of the species we observed, and experimentally by analysing the different fractions. We determined how total counts and filter mesh sizes affected the reliability of estimates of patterns of abundance, species richness and assemblage structure.

Site location

Lake Lautrey (46°35′14″N; 5°51′50″E) is located at 788 m above sea level in the Jura Mountains of eastern France (Fig. 1). Most of the lake has been filled with sediment through natural processes and nowadays it is reduced to a relatively small pond (75 m × 40 m) surrounded by mires. The lake is fed by small streams and runoff from an approximately 2 km^2 catchment area. The lake outlet falls into a karstic cavity to flow underground. The lake catchment area is hilly, culminating at about 830 m, with a forest cover dominated by *Abies* and *Fagus*. The bedrock is composed by Jurassic and Cretaceous limestone with outcrops of dolomite. The average monthly temperature ranges from −1°C in the coldest month to 16°C in the warmest month with mean annual precipitation approximately 1,500 mm. A sediment core (Fig. 2) was extracted from Lake Lautrey using a 10-cm diameter and 100-cm long Russian peat corer (Magny et al. 2006).

Stratigraphy of the sedimentary sequence

The stratigraphy of Core 6 (Fig. 2) was described by Magny et al. (2006) and is characterized by four sediment units, which generally correspond to Late glacial to early Holocene climatic phases in accordance with most of the abiotic indicators used for palaeoenvironmental studies. Sediment unit 1 (500–445 cm) is composed of dark-grey clayey silts and was assigned to the Oldest Dryas cold period. Sediment unit 2 (445–326 cm) is composed of yellow-green clayey biogenic carbonate lake marl from 445 to 430 cm, and yellow-beige silty biogenic

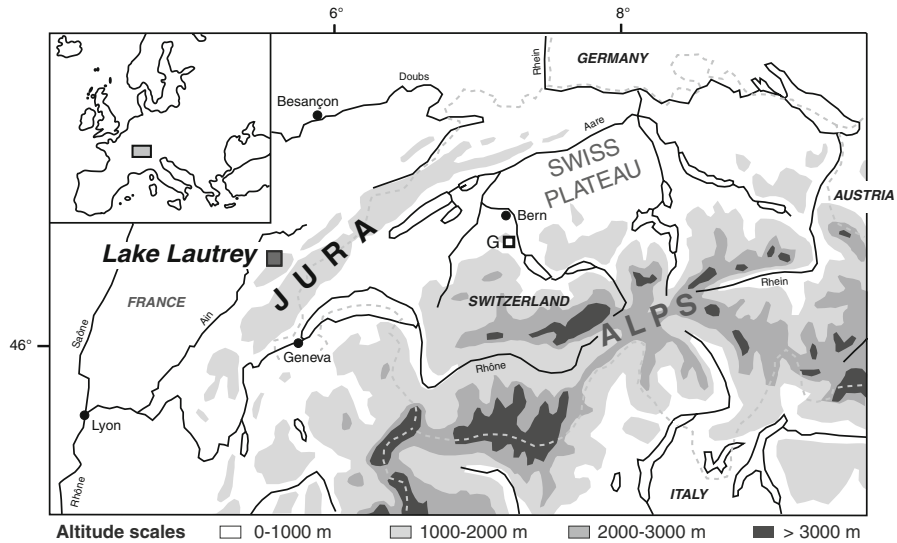


Fig. 1 Location of Lake Lautrey in west central Europe. G: Gerzensee

carbonate lake marl from 430 to 326 cm. This unit also includes the Laacher See Tephra (LST) at 345 cm. The Bølling/Allerød climatic temperate phase is represented by this part of the core. Sediment unit 3 (326–298 cm) is composed of grey clayey silts from 326 to 311 cm, and alternating layers of grey clayey silts and yellow-beige biogenic lake marl from 311 to 298 cm. This unit corresponds to the Younger Dryas cold period. Sediment unit 4 (298–229 cm) is composed of yellow-beige autogenic lake marl related to the Preboreal (early Holocene) temperate phase.

Methods

Seven sediment samples from this core were used in this study (Fig. 2). Three of them were taken from levels corresponding to cold climatic phases (samples 1–3) with the rest from warmer periods (samples 4–7). Two of the samples were constituted of clayey silts whereas the others were constituted of carbonate lake marl (Fig. 2). Since the goal of this study is not the palaeoenvironmental reconstruction, the six-first samples were numbered in ascending order of testate amoeba abundance rather than chronologically. The seventh sample was used in the second part of this study in order to test the effects of filter-mesh size independently from the six samples on which the first part of the study was done.

Testate amoeba analyses

For each sample, 0.3 cm^3 of sediment was taken and placed in a beaker with distilled water and agitated using a vortex during approximately 1 min to separate mineral particles from the shells. Each sample was then sieved on meshes of 250 and 25 μm. The <25-μm fraction of each sample was observed but no entire shells were found, just some fragments of *Paraquadrula irregularis* and *Difflugia* species. The remains from the fraction >250 μm were observed and just a few shells were noticed i.e. 2–3 shells per full sample (mostly shells of *Centropyxis aculeata* or *Difflugia oblonga*), representing less than 1% of the total sample count. Each sample was divided into fractions of equal volumes, which depending on the sample, represented between 9 and 14 fractions. Then, small quantities of liquid were transferred into a Hydro-Bios combined plate chamber (Utermöhl 1958).

Several sievings were done on equal-volume fractions of sample 7 using mesh-sizes of 47 and 63 μm, and each fraction was then counted separately. The objective was to compare the predicted effect of filter type on the estimated assemblage structure of sample 7 based on the shell sizes of individual species.

Testate amoebae were observed with an inverted optical microscope, (OLYMPUS IX71) for quantitative and qualitative analysis (Utermöhl 1958).

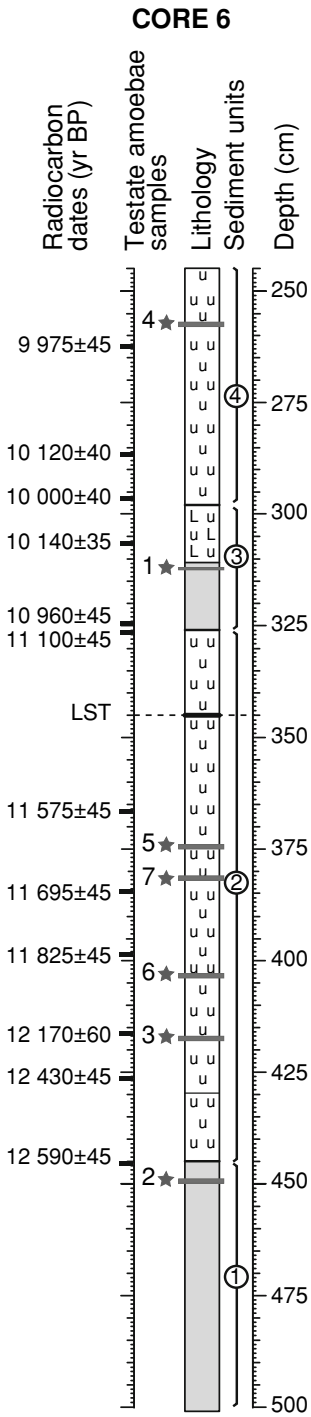


Fig. 2 Sediment sequence (Core 6) from Lake Lautrey (Jura, France) with stratigraphy according to Magny et al. (2006). Stars indicate the position of samples taken for testate amoeba analysis. Lithology: in grey colour = clayey silts; U = carbonate lake-marl; LU = clayey silts and carbonate lake-marl; LST = Laacher See Tephra

Samples 1–6 were counted in totality regardless of the density of shells. Testate amoebae were identified to the species level whenever possible. For sample 7, two fractions were counted in totality and the different size fractions (63–250, 47–63, and 25–47 μm) were counted separately. Only fragments corresponding to at least 50% of the entire shell were tallied to avoid double counting.

Images and morphometric measurements were used for species identification. The photos of specimens were examined and the most common form of each morphotype was compared with taxonomic monographs (Cash and Hopkinson 1905, 1909; Cash et al. 1915, 1919; Chardez 1967; Ogden and Hedley 1980; Ogden 1983; Meisterfeld 2002a, b). Some organisms could not be identified beyond the genus level because of the poor preservation of their shells.

Numerical analyses

Our first approach was to determine how filters with different mesh-sizes affected the estimates of density, species richness, diversity and equitability. To assess to what extent the use of 63-μm mesh might affect palaeoenvironmental interpretation; we first used a morphometrical approach and considered that the mesh sizes used for filtering the samples represented absolute thresholds. We thus compared the species richness and density of the fractions above and below 63-μm shell length. In a second step we compared the assemblage structure of sample 7 as assessed using 25-, 47-, and 63-μm mesh-size filters. Thus, we down weighted the abundance of each species from samples 1–6 according to the relative abundances recorded in the different size fractions of sample 7 (i.e. 25–47, 47–63, 63–250 μm). Then we compared the density, species richness, Shannon's H diversity and equitability in the full data set of such modified samples 1–6. The relative abundances of species absent in reference sample 7 were calculated by using another species of similar size and shape.

Our second approach was to assess the impact of total count size on species richness, or the appearance of new species. We calculated rarefaction curves for samples 1–6 using the PAST software version 1.57 (Hammer et al. 2001) with a randomisation of the data corresponding to 1,000 permutations proportionally calculated as a function of the relative abundance

of each species present in samples, with standard deviations and 95% confidence intervals. This analysis was first done by considering the total assemblage for each sample, then including only the species that reached 0.5–10% of relative abundance. These rarefaction curves were used in a second step to assess how many shells need to be counted in order to obtain reliable estimates of species representing 0.5–10% of the total assemblage. We also assessed the percentage of total species richness remaining when species representing 0.5–10% of the total assemblage are not included, and the relationship between species richness and the number of shells counted.

The third approach was to assess the combined effects of filter size and total count on estimates of total density, species richness, diversity and equitability. This was done by comparing the estimates based on the two fractions of sample 7 and by using a rarefaction analysis based on the total assemblage of samples 1–6, but down-weighting the abundance of each species according to the relative abundances recorded in the different fractions of sample 7 (i.e. 25–47, 47–63, 63–250 μm).

Spearman's rank correlation, Friedman and Wilcoxon tests were performed using the R software (R Development Core Team 2007).

Results

Abundance and species richness of testate amoebae

A total of 9,220 testate amoeba shells belonging to 43 taxa was counted in the >25-μm fraction on the first six samples (Tables 1, 2). The majority of these species belonged to Order Arcellinida, Kent 1880. The abundance of shells varied among samples between 421 and 3,261 (representing 1,403–10,870 shells cm^{-3}) and the species richness varied between 15 and 20 species (Tables 1, 2).

To test whether splitting the samples into subsamples could cause a bias in total abundance estimates, we plotted the cumulative number of testate amoebae observed as a function of sediment volume analysed (Fig. 3). The results show that there is no bias. For each sample a strong and highly significant linear relationship was observed ($R^2 > 0.99$; Spearman's rank correlation tests, $P < 0.0001$). Depending on samples, the number of sub-samples ranged from 8 to 14.

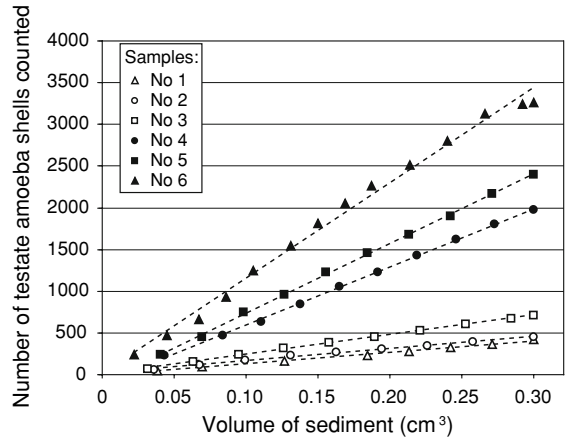


Fig. 3 Number of shells of testate amoebae counted in relation to the volume of sediment analysed (cm^3) for samples 1–6 from Lake Lautrey. Linear regression: $R^2 > 0.99$ and Spearman's rank correlation are highly significant ($P < 0.0001$). *Full symbols* indicate high densities samples and open ones low densities samples

Testate amoeba assemblages and impact of filter mesh size

The most abundant species were, in decreasing average abundance, *Paraquadrula irregularis, Centropyxis aculeata, Centropyxis ecornis, Difflugia* type *olliformis, Centropyxis constricta, Phryganella acropodia* and *Centropyxis discoides* (Table 1). For samples 1–6, species smaller than 63 μm in length and width made up between 29.0 and 89.0% of the total counts and represented between 26.3 and 42.9% of the species richness (Table 2).

A total of 404 shells representing 18 species were counted in sample 7 when the results of the three fractions are added. Of these, 198 shells and 8 species were counted in the 25–47-μm fraction, 89 shells and 11 species in the 47–63-μm fraction, and 117 shells and 18 species in the 63–250-μm fraction. Species present in the <63-μm and <47-μm fractions represented, respectively, 71 and 49% of the total counts. Using different mesh sizes also significantly affected the perceived assemblage structure of the sample (Fig. 4). With a 63-μm mesh size *Centropyxis* species made up 47% of the assemblage and *Difflugia* species 33.4%. *Paraquadrula irregularis* made up only 14.5% of the assemblage. However, according to its size, this

Table 1 Results of testate amoebae analysis of six samples from Lake Lautrey

						sample n°1			sample n°2			sample n°3			sample n°4			sample n°5			sample n°6		
Species <63 µm on average	L		l		n	N	ind.cm^{-3}	%	N	ind.cm^{-3}	%	N	ind.cm^{-3}	%	N	ind.cm^{-3}	%	N	ind.cm^{-3}	%	N	ind.cm^{-3}	%
Arcella gibbosa	55		49		1																36	120.0	1.10
Centropyxis aerophila	63	*(46-72)*	48	*(40-58)*	8				18	60.0	4.03	27	90.0	3.80	1	3.3	0.05	6	20.0	0.25			
Cyclopyxis eurystoma	59	*(51-69)*	45	*(30-55)*	7	11	36.7	2.61							1	3.3	0.05				14	46.7	0.43
Difflugia glans	52		47		1										43	143.3	2.17						
Difflugia globulosa	52	*(45-61)*	49	*(44-54)*	7				84	280.0	18.79	15	50.0	2.11									
Difflugia penardi	54	*(47-59)*	44	*(33-54)*	10				10	33.3	2.24												
Difflugia type olliformis	60	*(48-77)*	48	*(37-61)*	15	10	33.3	2.38							51	170.0	2.58	100	333.3	4.16	302	1,006.7	9.26
Diplochlamys timida	45	*(42-49)*	39	*(35-46)*	3				51	170.0	11.41												
Paraquadrula irregularis	42	*(31-55)*	40	*(30-55)*	15	38	126.7	9.03				356	1,186.7	50.14	1,116	3,720.0	56.39	1,750	5,833.3	72.86	2,445	8,150.0	74.98
Phryganella acropodia	44	*(32-54)*	41	*(30-52)*	12	43	143.3	10.21	8	26.7	1.79	54	180.0	7.61	33	110.0	1.67	86	286.7	3.58	36	120.0	1.10
Phryganella sp2	62	*(50-75)*	55	*(46-73)*	5							4	13.3	0.56				62	206.7	2.58			
Pseudodifflugia type horrida	54	*(37-58)*	51	*(37-57)*	8	20	66.7	4.75							18	60.0	0.91	11	36.7	0.46	69	230.0	2.12
Total						**122**	**406.7**	**28.98**	**171**	**570.0**	**38.26**	**456**	**1,520.0**	**64.23**	**1,263**	**4,210.0**	**63.82**	**2,015**	**6,716.7**	**83.89**	**2,902**	**9,673.3**	**88.99**
Species with width <63 µm																							
Centropyxis sp.	75	*(72-89)*	62	*(47-79)*	7				9	30.0	2.01												
Difflugia elegans	82		61		2													2	6.7	0.08	3	10.0	0.09
Difflugia mamillaris	105		61		1	1	3.3	0.24															
Difflugia manicata	76		55		1	2	6.7	0.48															
Heleopera sp.	81	*(74-85)*	54	*(46-61)*	3				11	36.7	2.46	4	13.3	0.56	18	60.0	0.91						
Hyalosphaenia elegans	91	*(87-94)*	45	*(40-52)*	3										1	3.3	0.05						
Total						**3**	**10.0**	**0.71**	**20**	**66.7**	**4.47**	**4**	**13.3**	**0.56**	**19**	**63.3**	**0.96**	**2**	**6.7**	**0.08**	**3**	**10.0**	**0.09**
Other species																							
Arcella discoides	160	*(108-196)*	159	*(108-193)*	3				1	3.3	0.22												
Arcella vulgaris	102	*(76-139)*	97	*(71-133)*	15																9	30.0	0.28
Centropyxis aculeata	104	*(83-137)*	84	*(64-108)*	15	40	133.3	9.50	19	63.3	4.25	53	176.7	7.46	292	973.3	14.75	184	613.3	7.66	98	326.7	3.01
Centropyxis constricta	98	*(73-128)*	77	*(61-98)*	15	45	150.0	10.69	85	283.3	19.02	44	146.7	6.20	79	263.3	3.99	83	276.7	3.46	30	100.0	0.92
Centropyxis discoides	170	*(126-220)*	153	*(110-215)*	16	35	116.7	8.31				85	283.3	11.97	97	323.3	4.90	11	36.7	0.46	9	30.0	0.28
Centropyxis ecornis	125	*(105-144)*	106	*(85-134)*	36	76	253.3	18.05	84	280.0	18.79	46	153.3	6.48	116	386.7	5.86	73	243.3	3.04	70	233.3	2.15
Centropyxis platystoma	82	*(69-89)*	64	*(48-71)*	4				8	26.7	1.79												
Cyclopyxis kahli	92	*(76-118)*	87	*(72-116)*	20	45	150.0	10.69	30	100.0	6.71	8	26.7	1.13	17	56.7	0.86	24	80.0	1.00			
Difflugia ampullula	82	*(59-97)*	74	*(56-91)*	20	28	93.3	6.65							41	136.7	2.07	8	26.7	0.33	67	223.3	2.05
Difflugia avellana	113	*(87-141)*	78	*(62-100)*	15																1	3.3	0.03
Difflugia curvicaulis	113	*(62-153)*	72	*(61-84)*	4	1	3.3	0.24															
Difflugia globularis var. microstoma	79	*(54-102)*	71	*(51-85)*	9																14	46.7	0.43
Difflugia lithophila	115		96		1	3	10.0	0.71															
Difflugia oblonga nodosa	172	*(111-250)*	121	*(96-193)*	10	16	53.3	3.80													6	20.0	0.18
Difflugia parva	128	*(101-160)*	93	*(79-111)*	5										6	20.0	0.30						
Difflugia type decloitrei	88	*(72-117)*	73	*(62-101)*	3										27	90.0	1.36						
Difflugia type gramen	67		64		2				3	10.0	0.67												
Difflugia type labiosa	131		81		1				1	3.3	0.22												
Difflugia type oblonga	265		126		1							1	3.3	0.14									
Difflugia type rotunda	137	*(124-173)*	126	*(110-168)*	6	7	23.3	1.66	4	13.3	0.89	2	6.7	0.28									
Heleopera petricola	82	*(56-108)*	68	*(50-95)*	11				11	36.7	2.46	6	20.0	0.85	22	73.3	1.11	2	6.7	0.08			
Heleopera rosea	118	*(103-134)*	77	*(60-93)*	5				9	30.0	2.01												
Lesquereusia spiralis	97		84		1				1	3.3	0.22												
Phryganella sp1	102		99		2																52	173.3	1.59
Pontigulasia type elisa	99		76		2							5	16.7	0.70									
Total						**296**	**986.7**	**70.31**	**256**	**853.3**	**57.27**	**250**	**833.3**	**35.21**	**697**	**2,323.3**	**35.22**	**385**	**1,283.3**	**16.03**	**356**	**1,186.7**	**10.92**
TOTAL						421	1,403.3	100	447	1,490.0	100	710	2,366.7	100	1,979	6,596.7	100	2,402	8,006.7	100	3,261	10,870.0	100

Number of shells counted (*N*), number of shell cm^{-3}, and percentage for each species. An average of the lengths (*L*) and widths (*l*) based on several (*n*) measurements is given. The first group of species is on average smaller than 63 µm in length. Species from the second are on average longer than 63 µm but narrower than 63 µm. Species from the third group are on average both longer and wider than 63 µm

Table 2 Summary of the shell density and species richness (actual values and percentages) of testate amoebae larger and smaller than 63 μm observed in samples 1–6 from Lake Lautrey

	Total abundance					Species richness				
	sp > 63 μm		sp < 63 μm			sp > 63 μm		sp < 63 μm		
Sample	*N*	%	*N*	%	Total	*N*	%	*N*	%	Total
1	299	71.02	122	28.98	421	12	70.59	5	29.41	17
2	276	61.74	171	38.26	447	14	73.68	5	26.32	19
3	254	35.77	456	64.23	710	10	66.67	5	33.33	15
4	716	36.18	1,263	63.82	1,979	11	61.11	7	38.89	18
5	387	16.11	2,015	83.89	2,402	8	57.14	6	42.86	14
6	359	11.01	2,902	88.99	3,261	11	64.71	6	35.29	17

species should theoretically be lost, but a fraction of the individuals were retained by the larger mesh-size filters, probably due to clogging of the filter or adhesion to larger particles. With a mesh size of 47 μm the proportion of *Centropyxis* species decreased and that of *Difflugia* species slightly increased (i.e. respectively, 36 and 37.9%) while the proportion of *Paraquadrula irregularis* increased to 19.4%. Shannon diversity and the equitability values were similar for the 63-and 47-μm mesh sizes. Shannon'H values were respectively, 2.56 for the 63-μm and 2.45 for 47-μm mesh sizes, and the equitability was about 0.89 for the 63 μm and 0.86 for 47-μm mesh sizes. With a mesh size of 25 μm, *P. irregularis* alone dominated the assemblage with 50.5% while *Centropyxis* species and *Difflugia* species only contributed to 18.9 and 23% of the assemblage, respectively. This resulted in lower values for Shannon diversity (1.90) and equitability (0.66).

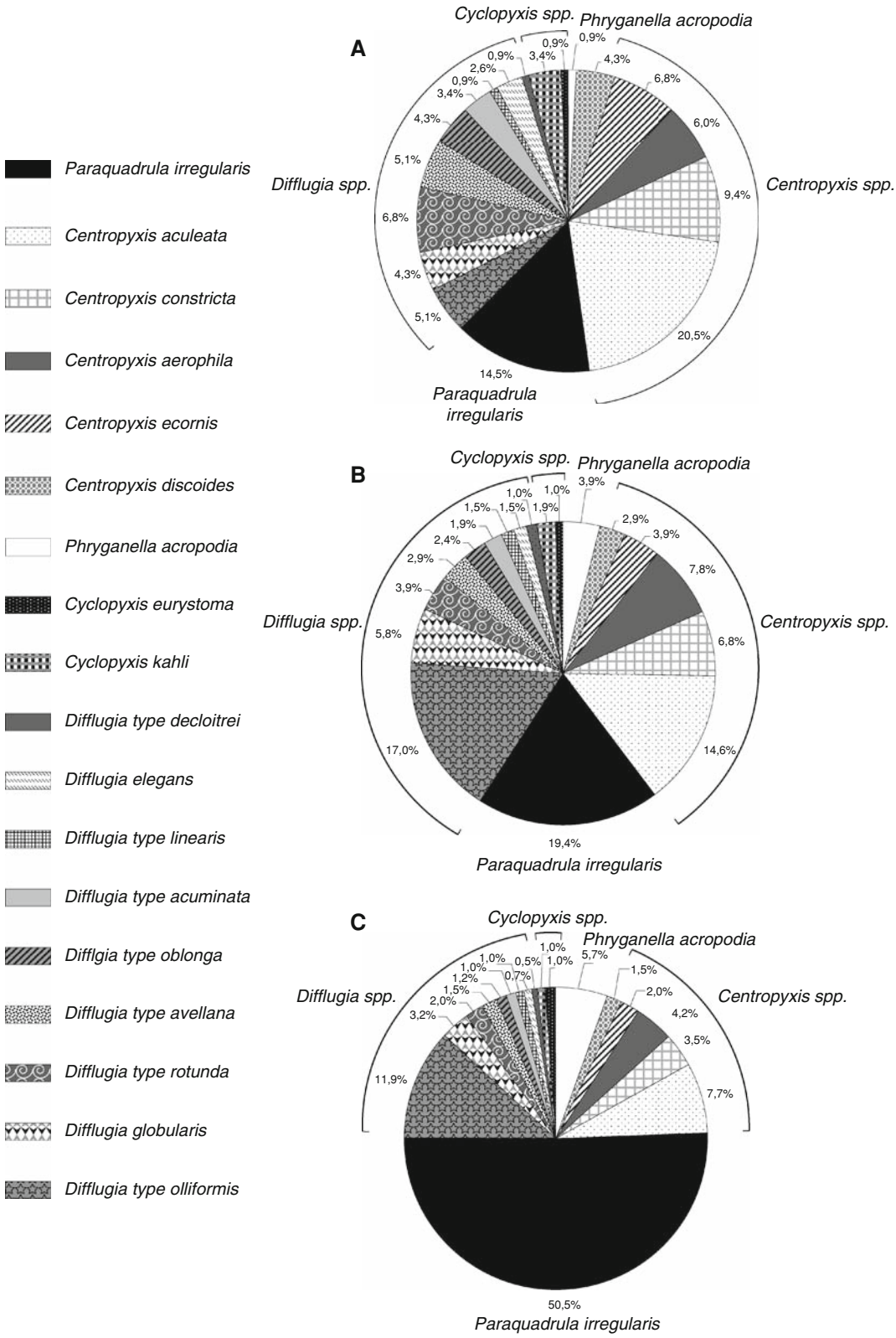
Figure 5 shows how the density, species richness, Shannon's *H* diversity and equitability vary in samples 1–6 for different scenarios corresponding to the effect of using different filter mesh sizes for sample 7. This analysis revealed that using the whole data (>25 μm), the density of samples 1–3 stood out as being much lower than that of samples 4–6, but this difference between samples was gradually lost when larger mesh sizes were used to filter the sample (pairwise comparisons Wilcoxon tests: $W = 21$, $z = 2.201$, $P = 0.028$ for the three filtrations). Likewise, the clear differences in diversity and equitability observed on the >25-μm fraction were lost when coarser filters were used. Estimates of species richness were biased when a total of 160 shells and a 63-μm mesh were used, whereas no bias was observed with a total of 404 individuals and/or a smaller mesh of 47 μm.

Impact of total count on species richness

Rarefaction curves for samples 1–6 were built with a confidence interval of 95%, respectively, for the totality of the shells from each sample, and for relative abundances lower than 0.5, 1, 1.5, 2, 2.5, and 3–10%. Examples of those curves were given in Fig. 6 for relative abundances lower than 1, 2, 4, 7, and 10%.

Based on these curves, we assessed how many shells should be counted in order to obtain reliable estimates of species representing 0.5–10% of the total assemblage (Fig. 7A). This analysis showed that, on average over the six samples, if 400 shells were counted, species representing as little as 2% of the total assemblage would be recovered. If the aim is to obtain information only on species representing 4% or more of the assemblage, then a total count of 170 shells is sufficient. This relationship is clearly non-linear.

The loss of rare species caused the total species richness to be underestimated, and this relationship was non-linear (Fig. 7B). For total count of 170 shells, if only species representing more than 4% of the total assemblage were included only 31% of the total species richness would be recovered on average. If species representing as little as 2% of the total count were included, which would require on average a count of 400 shells, then half of the total species richness could still be missed.


Paraquadrula irregularis
Centropyxis aculeata
Centropyxis constricta
Centropyxis aerophila
Centropyxis ecornis
Centropyxis discoides
Phryganella acropodia
Cyclopyxis eurystoma
Cyclopyxis kahli
Difflugia type decloitrei
Difflugia elegans
Difflugia type linearis
Difflugia type acuminata
Difflgia type oblonga
Difflugia type avellana
Difflugia type rotunda
Difflugia globularis
Difflugia type olliformis
A
Cyclopyxis spp.
Phryganella acropodia
Difflugia spp.
Centropyxis spp.
Paraquadrula irregularis
0,9%
0,9%
3,4%
0,9%
2,6%
0,9%
3,4%
4,3%
5,1%
6,8%
4,3%
5,1%
14,5%
20,5%
9,4%
6,0%
6,8%
4,3%
B
Cyclopyxis spp.
Phryganella acropodia
Difflugia spp.
Centropyxis spp.
Paraquadrula irregularis
1,0%
1,0%
1,5%
1,5%
1,9%
1,9%
2,4%
2,9%
3,9%
5,8%
17,0%
19,4%
14,6%
6,8%
7,8%
3,9%
2,9%
3,9%
C
Cyclopyxis spp.
Phryganella acropodia
Difflugia spp.
Centropyxis spp.
Paraquadrula irregularis
1,0%
1,0%
0,5%
1,0%
1,0%
0,7%
1,2%
1,5%
2,0%
3,2%
11,9%
50,5%
7,7%
3,5%
4,2%
2,0%
1,5%
5,7%

◀**Fig. 4** Relative abundance of testate amoeba taxa in sediment sample 7 from Lake Lautrey using filters of three different mesh-sizes: **A** 63 μm, **B** 47 μm, **C** 25 μm. The 3 assemblage structures are significantly different (Friedman $X^2 = 20.83$, $P < 0.001$; Wilcoxon tests for pairwise comparisons: W (25 μm/47 μm) = 36, $z = 2.54$, $P = 0.011$; W (25 μm/63 μm) & (47 μm/63 μm) = 66, $z = 2.94$, $P = 0.003$)

Results from rarefaction curves for samples 1–6 showed that on average a total of only 100 shells might suffice to recover more than 50% of the species (Fig. 8). However, this percentage varied strongly among samples from a minimum of 40.8% in sample 6 to a maximum of 95.6% in sample 1. The usual total of 150 shells allowed recovering at least 50% and up to 99.5% of the species richness. A count of 170 shells yielded more than 68% of the species richness, and a count of 400 shells more than 82%.

Finally, total count and filter mesh size interacted in modifying the perceived species richness of samples (Fig. 8). Interestingly this effect was not the same on all samples; it was strongest in samples 5 and 6 that were strongly dominated by *Paraquadrula irregularis*. Thus in these cases, the estimates of species richness would be better if small and dominant species were mostly lost during sample preparation. The reverse situation was observed, albeit much less clearly, for samples 1 and 2, in which no small species was dominant and which had a more equitable assemblage.

Discussion

Overall abundance in relation to sample volume

A high statistical significance of Spearman's rank correlations for the number of shells with the sediment volume (Fig. 3) shows that subsamples are homogenous and are representative of the total abundance of testate amoeba from the sample. In this study, relatively small sample volumes (0.3 cm^3) clearly yielded sufficient numbers of testate amoeba shells to evaluate abundance and species richness, as long as the majority of the assemblage was taken into account, and not only the larger-size fraction (>63 μm). However, sediment volumes of 5.5–10 cm^3 are usually processed in palaeolimnological studies (Beyens and Meisterfeld 2001). Thus, the first implication of our results is that it is possible to obtain sufficient testate amoeba data for palaeolimnological studies using very small sample volumes. Small sample volumes also allow very thin layers of sediment to be used, thus achieving higher

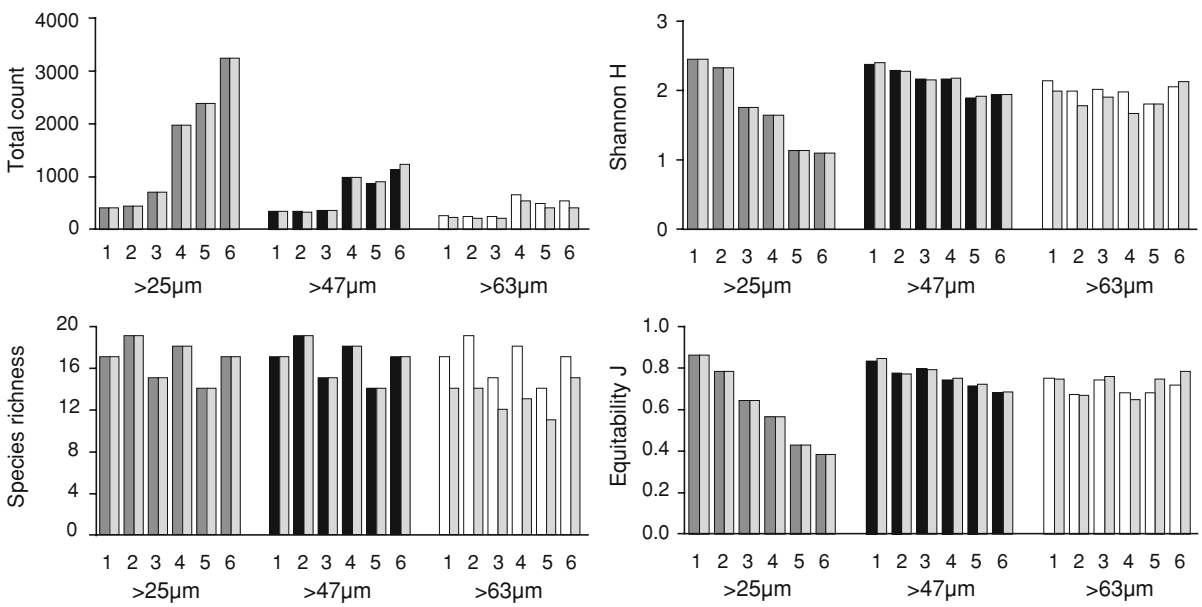


Fig. 5 Testate amoeba density, species richness, Shannon H diversity and equitability in sediment samples 1–6 from Lake Lautrey. Dark grey bars = full data set (>25 μm) for 404 shells counted from sample 7; black bars = species with shell length < 47 μm excluded; white bars = species with shell length < 63 μm excluded. The *light grey* series represents the same calculations for a total count of 160 shells

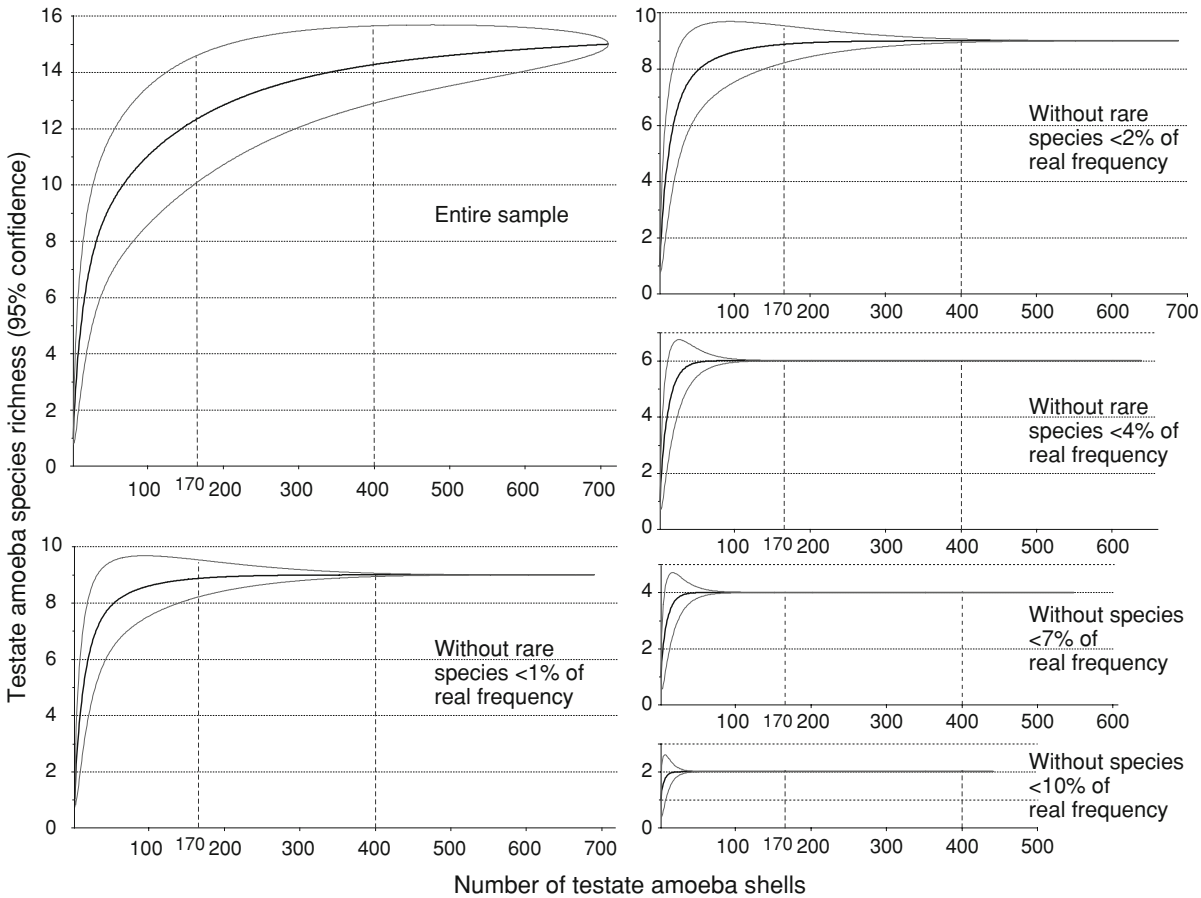


Fig. 6 Rarefaction curves from a randomisation of sample number 3 representing the variation of testate amoeba species richness with 95% confidence intervals as a function of the number of shells counted. The *graphs* are given for the total assemblage and for reduced data sets in which species whose relative abundance stand below 1, 2, 4, 7, and 10% were removed

temporal resolution. However, if coarse mesh sizes are used, larger volumes of sample are required, thus limiting the potential temporal resolution of the study for any given core diameter.

Species richness, abundance, and assemblage structure of testate amoebae in relation to mesh size used

Most testate amoeba species observed in this study are commonly found in studies of lacustrine sediments (McCarthy et al. 1995; Asioli et al. 1996; Dallimore et al. 2000). The relative abundances of species differ among studies, but the number of shells counted and the number of species are generally similar.

However, several species, such as *Paraquadrula irregularis*, *Difflugia* type *olliformis* and *Diplochlamys timida*, were hardly ever reported in lake sediments. The standard sample preparation of lacustrine samples using a >63 μm filtration (Medioli and Scott 1983; Scott and Medioli 1983; Patterson et al. 1985, 1996) most likely explains why species like *P. irregularis* were not recorded in most previous studies. However, the absence of those species in other studies can be related to variations in determination or ecology, for example, Schönborn (1973, 1984) frequently found other species of *Paraquadrula* and several small species. Twelve of the 43 testate amoeba species recorded are smaller than 63 μm, and the total would be 18 species if shell width and not length is considered (Table 1). These

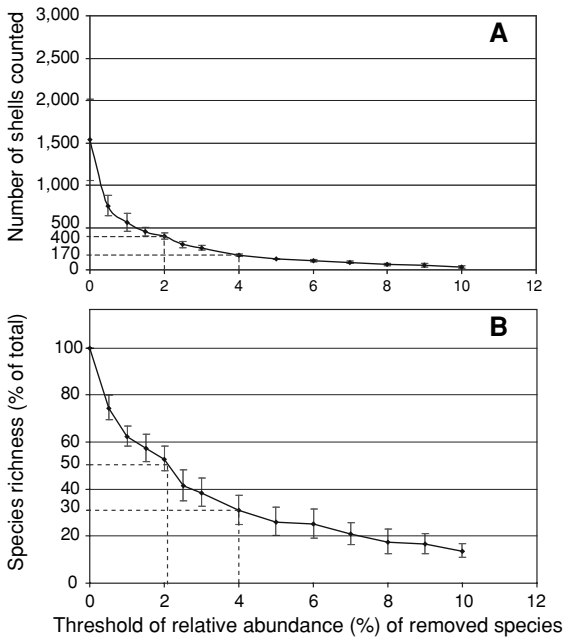


Fig. 7 **A** Estimated number of shells that need to be counted in order to reach good estimate of testate amoeba species richness based on the results of the rarefaction analysis. *Dotted lines* correspond to total counts of 170 and 400 shells respectively, after removing species with relative abundances below 2 or 4%. **B** Estimates of the losses in terms of number of species (% of total) based on the rarefaction analysis. *Dotted lines* show cases corresponding to (1) 50% of total species richness and (2) the species richness percentage corresponding to the case when species with relative abundance below 4% are excluded. The *points* represent average values for all samples and *bars* indicate standard errors

taxa include the most abundant species in this study, *P. irregularis*, and several other common species such as *Phryganella acropodia*, *Centropyxis aerophila*, *Difflugia* type *olliformis* or *Pseudodifflugia* type *horrida* (Table 1). All these species will at best be underestimated or more likely totally overlooked using 63 μm mesh filters (Charman et al. 1998).

According to our calculations, excluding the smaller testate amoeba species would reduce the total abundance and species richness (Table 2). The use of a 63-μm mesh therefore clearly leads to biased estimations of density and species richness and may cause significant changes in assemblages to be overlooked, leading to erroneous palaeoecological information. This theoretical bias due to the use of large mesh sizes was confirmed by the comparative study of sample 7 using mesh sizes of 63, 47, and 25 μm. The relative abundance of *Paraquadrula irregularis* increased from 14.5 to 50.5% in these three fractions (Fig. 4). Furthermore species present in the <63- and <47-μm fractions represented, respectively, 71 and 49% of the total counts. These fractions of the total abundance of shells would therefore be missed by using these mesh sizes for counts.

Our calculations based on shell sizes are perhaps conservative in that species longer but narrower that 63 μm were not considered in the calculations even if they can theoretically pass through the mesh of the 63-μm filter and therefore can also be lost. On the other hand, the comparative study of sample 7 showed that some smaller species were still recovered using the larger mesh, but only a fraction of them, thus yielding erroneous estimates of assemblage composition (Fig. 4). Using coarser mesh sizes also induced biases in estimates of density, species richness, diversity and equitability estimates (Fig. 5), which may change the interpretation of results.

Charman et al. (1998) conducted a comparative analysis of different sample preparation methods using material collected in estuarine habitats. They observed a 18-fold change in species richness, from two species in the >63-μm fraction to 36 species in the <63-μm fraction. This clearly illustrated the importance of including the smaller fraction for ecological and palaeoecological studies of estuaries. Our study shows that mesh size is also critical in lake studies.

Beyens and Meisterfeld (2001) recommend the use of a 10–15 μm screen to recover most of the species diversity. In our study, the smaller fractions (<25 μm) did not contain any testate amoebae, suggesting that a 25-μm mesh size may be sufficient to recover the full species richness while still removing many smaller mineral particles from the lacustrine sediments, thus making counting an easier process.

In order to correctly assess testate amoeba assemblage structure, it is important to have reliable estimates of both species richness and abundance of shells from each species. The results suggest that the commonly used methodology should be reconsidered if obtaining estimates of species richness, density, and whole assemblage structure is important for the research questions addressed.

Total count and estimations of rare species

Counting fossil amoeba shells is time consuming and the current standard is to count at least 150 shells

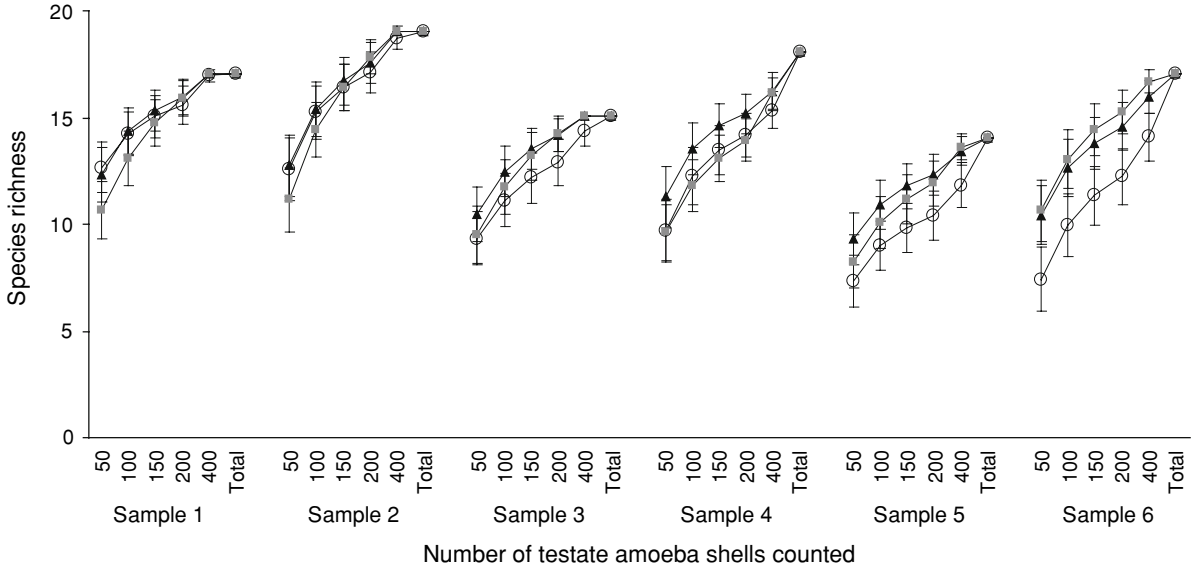


Fig. 8 Number of species recovered on the full sample (>25 μm, *white open circles*), >47 μm fraction (*black triangles*), and >63 μm fraction (*grey squares*) for different total number of testate amoeba shells counted for samples 1–6 from Lake Lautrey. The *points* represent average values over 1,000 random from rarefaction analyses for the sample and standard deviations. Values for the >47 and >63 μm fraction are based on the >25 μm fraction and estimates of species richness loss from sample 7 (Fig. 4 and text)

(Beyens and Meisterfeld 2001). This study shows that the number of shells to be counted depends on the nature of sediment used and the aim of the study (Table 3). According to results of rarefaction analysis (Figs. 6, 7), a count of 400 shells allows certain recovery of species with a relative abundance above 2% of the total species present in the sample. A count of 170 shells is sufficient if the goal is to find species with relative abundances higher than 4%. This relationship is not a strict one. For example, with a total count of 400 shells, some rare species, in this case below 2% of relative abundance, will be found, but the abundance estimates for these species will not be reliable.

With a total count of 100 shells, theoretically more than 50% of the total species richness can be recovered, independent of the origin of the sample in terms of climatic period and nature of the sediment (Fig. 8). Thus, 100 shells could describe the general testate amoeba assemblage structure for samples with low densities of shells, whereas a somewhat higher total count would be necessary for samples with higher densities. But depending on the research question 50% of the species richness may not be sufficient. When rare species are not recovered owing to low total count, the estimated total species richness is clearly reduced. According to the results of rarefaction analyses (Fig. 7B) even when species representing as little as 2% of the total assemblage are recovered accurately, with counts of 400 individuals, about half of the total species richness may be overlooked.

Since the ecology of many testate amoeba species remains unknown, it is difficult to assess the bioindicator value of these rare species and hence if it is worth spending time to recover them. Indeed Payne and Mitchell (2009) recently showed that for peat samples the dominant species may contain sufficient information for inferring the main ecological shifts, and that time might thus be much better invested in counting more samples but with low counts (e.g. as little as 50 individuals) than obtaining precise estimates of rare species. In addition, some very rare species may simply represent accidental species that have been washed into the lake. In this case not only would they not bring much useful information, but worse they may indeed add noise to the data. Unless this accidental input is indicative of some important processes occurring in the catchment such as increased erosion due to overgrazing or drought. Further studies are clearly needed to determine the ecological role and/or bioindicator value of these rare species.

Table 3 Main conclusions of different figures and tables of this study in terms of loss in percentages function of total testate amoebae shells counted and mesh size of filter

	Effect of total count on:	Total number of shells counted		
		100	170	400
Figure 7	Threshold of species' relative abundance (%) below which estimates are unreliable[a]	6 %	4 %	2 %
	Percentage of the total species richness represented by these species:			
	- for all samples (1-6)	74.9% (SE= 6.3%)	69.1% (SE= 6.2%)	47.2% (SE= 5.2%)
	- for low density samples (1-3)	62.2% (SE= 6.1%)	56.7% (SE= 4.9%)	37.6% (SE= 5.0%)
	- for high density samples (4-6)	87.6% (SE= 1.0%)	81.5% (SE= 3.4%)	56.8% (SE= 4.3%)
Figure 8	Loss of species richness based on rarefaction randomisation of the full samples (1000 permutations):			
	- for all samples (1-6)	29.0% (SE= 4.0%)	20.6% (SE= 3.5%)	9.3% (SE= 3.2%)
	- for low density samples (1-3)	21.1% (SE= 2.9%)	13.3% (SE= 2.0%)	2.4% (SE= 1.3%)
	- for high density samples (4-6)	37.0% (SE= 2.8%)	27.9% (SE= 2.2%)	16.2% (SE= 0.7%)
	Effect of filter size on:	Mesh-size of filter 25µm	47µm	63µm
Table 1 & 2	Abundance:			
	- for all samples (1-6)		50% (SE= 11.3%)	61% (SE= 9.8%)
	- for low density samples (1-3)	n.a. (whole sample)	30% (SE= 14.0%)	44% (SE= 10.5%)
	- for high density samples (4-6)		70% (SE= 6.1%)	79% (SE= 7.7%)
	Species richness:			
	- for all samples (1-6)		12% (SE= 0.6%)	34% (SE= 2.5%)
	- for low density samples (1-3)	n.a. (whole sample)	12% (SE= 0.8%)	30% (SE= 2.0%)
	- for high density samples (4-6)		12% (SE= 1.0%)	39% (SE= 2.2%)
Figure 4	Filtration effect on sample 7: dominant species with relative abundances >10%	*P. irregularis* (50.5%) *D. cf olliformis* (11.9%)	*P. irregularis* (19.4%) *D. cf olliformis* (17.0%) *C. aculeata* (14.6%)	*C. aculeata* (20.5%) *P. irregularis* (14.5%)
	Overall effect on assemblage structure	Friedman X^2 = 20.83, df = 2, p < 0.001		
	Pairwise comparisons (Wilcoxon tests)	W (25µm / 47µm) = 36, z = 2.54, p = 0.011 W (25µm / 63µm & 47µm / 63µm) = 66, z = 2.94, p = 0.003		
	Effect of filter size and total count on:			
Figure 5	Sample 7: Abundance	Significantly different for the 3 filtrations for both 160 and 404 totals counts		
	Species richness; total count 160	Filtration on 63µm significantly different from the other two because small species are recovered occasionally		
	Species richness; total count 404	No significant difference among the three filtrations because even small species are recorded		
	Shannon diversity and Equitability	Differences observed in >25µm fraction are gradually lost with larges mesh-sizes.		
Figure 8	Sample 1-6: species richness	Although in absolute terms the estimates of species richness will be closer to the true value using the coarsest mesh-size at low count totals the differences among samples will be biased		

[a]Species rarer than this threshold may be recovered but their occurrence and relative abundance estimated will not be reliable.

Total count and filter mesh size interact in affecting the perceived species richness and differences will be greatest for samples that are strongly dominated by small species. Figures 5 and 8 show that although in absolute terms the estimates of species richness will be closer to the true value using the coarsest mesh size at low count totals, the differences among samples will be biased.

Conclusion

This study showed that testate amoebae are abundant in lake sediments and that a sediment volume of 0.3 cm^3 is sufficient to obtain good estimates of assemblages in lacustrine deposits. The total abundance, species richness, assemblage structure and especially the proportion of dominant taxa observed are not well estimated using filters of 63 μm or even 47-μm mesh. Based on these results, we suggest that sediment samples should be sieved using a 25-μm mesh filter in order to retain, and accurately estimate the density of, the smaller species that dominate the assemblages.

Counts of 400 shells are needed to accurately estimate the abundance of rare species that represent approximately 2% of the total assemblage, but even with this total, half of the species richness may still be overlooked. Where densities are high, such as in samples from warmer climates, higher counts are needed to obtain reliable estimates because of the strong dominance of a single taxon, such as *Paraquadrula irregularis* in this study.

Unless the bioindicator value of rare species is established, it may be more time effective to count 170 shells and to increase the number of samples, and thus the temporal resolution. If small and/or rare species are not essential for palaeoenvironmental reconstructions, then larger mesh sizes would be preferable as most fine materials are removed, thus speeding up the counting process. Furthermore, low total counts will theoretically yield reliable data because the total potential species richness will be lower. However, this study shows that a few individuals of species smaller than the mesh size will still be recovered, potentially causing a bias in the difference of species richness estimates among samples. As the density estimate of these small species will not be accurate, it may be advisable to exclude them from the interpretation if coarse mesh-size filters are used.

Thus, the total number of shells that need to be counted is a function of the mesh size used. If a case can be made, as our data suggest, that small species provide important palaeoenvironmental information, then higher counts are needed to achieve reliable estimates of assemblage structure. If large species suffice, perhaps in a different ecological context than our study, or if the purpose is to address questions for which utilizing large species has been shown to be sufficient, then larger mesh sizes and lower counts could be used.

This study gives an idea of potential losses of species and errors in estimates of relative abundances of species using different filter sizes. It would be advisable to do comparable analyses at the beginning of each limnological and palaeolimnological study to determine the optimal analytical protocol.

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