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Testate Amoebae as Sea-level Indicators in Northwestern Norway: Developments in Sample Preparation and Analysis

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Abstract. Proxy based sea-level reconstructions are an important tool for defining past patterns of sea-level change and salt-marsh testate amoebae are a newly emerging proxy with high potential as sea-level indicators. This study develops existing analytical techniques concerned with the preparation and counting of testate amoebae for sea-level studies and demonstrates the predictive power of this group of micro-organisms. Two salt marshes in northwestern Norway were sampled for testate amoebae and multiple sub-samples were prepared using different procedures and count totals. Analytical efficiency can be improved upon by using a mild alkali, chemical disaggregant (5% KOH) to break up fibrous, salt-marsh peat and concentrate tests prior to counting. A count total of 100 individuals, rather than 150, can be used to make time gains with little or no loss of taxon information. The Norwegian salt-marsh testate amoebae showed strong zonation relative to tidal elevation. Key indicator taxa from the high marsh included *Centropyxis cassis* type, *Cyclopyxis arcelloides* type and *Euglypha* spp. Those from the low marsh included *Difflugia pristis* type and a distinctive morphotype of *Centropyxis platystoma* type. Combined, the two surface data sets from Norway were capable of predicting marsh surface elevations to within ± 0.09 m.

Key words: Salt marsh, testate amoebae, sea level, transfer function, Norway, preparation.

INTRODUCTION

Testate amoebae have been used as palaeoenvironmental indicators in peat and lake sediments for over 100 years (Tolonen 1986). In lakes they have been shown to be sensitive to lake water quality and base status, and in peatlands they have been extensively used as indicators of moisture status and hydrochemistry (Charman 2001, Mitchell *et al*. 2008). In neo-ecological studies they have also been shown to be responsive to factors such as air pollution (Nguyen-Viet *et al*. 2004), suggesting they have great potential as bio-indicators in a very wide range of settings. Whilst testate amoebae are abundant in terrestrial and freshwater habitats, they also occur in mildly saline soils such as those found in the higher areas of salt marshes, or enclosed lagoonal systems (Lloyd 2000). This has led to a realisation that fossil testate amoebae could be used as a sea-level indicator in salt-marsh sediments (Charman *et al*. 1998).

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Sea-level changes can be reconstructed from salt-marsh sediments by studying the zonation of micro-organisms across the marsh surface from high to low tide, and quantifying the surface elevations of these zones (Scott and Medioli 1978, 1980). Surface elevation determines the extent and duration of tidal flooding for each location, which, in turn, drives both moisture status and salinity, and species assemblages are determined by species turnover along this environmental gradient. An 'indicative meaning' for position along the elevational gradient can be assigned to particular assemblages or taxa and then applied to fossilised assemblages to estimate the surface elevation, relative to sea level, at the time of deposition. The indicative meaning of an indicator defines its environmental range with reference to a water level such as mean sea level (Shennan 2007). Sea-level studies using salt-marsh sediments have most commonly made use of foraminifera (e.g. Scott and Medioli 1982, Gehrels *et al*. 2006a) and diatoms (e.g. Szkornik *et al*. 2006, Woodroffe and Long 2009) to reconstruct past changes in sea level, but studies using testate amoebae are still sparse.

Testate amoebae were first systematically recorded in salt-marsh samples prepared for foraminiferal analysis (e.g. Scott and Martini 1982) and, by the mid-1990s, there was growing evidence that, when combined with foraminiferal data, testate amoebae fitted into a vertical zonation sequence, being indicative of upper marsh conditions (e.g. Scott *et al*. 1991, 1995). However, preparation of samples for foraminifera analyses was based on sieving only a relatively large size fraction (> 63 μm), so that the majority of the tests were being lost. Including smaller size fractions (> 15 μm) in counts revealed larger numbers of tests, greater taxon diversity (Charman *et al.* 1998), and a strong vertical zonation of assemblages across the marsh surfaces at a number of locations in the UK (Charman *et al*. 1998, 2002; Gehrels *et al*. 2001) and North America (Gehrels *et al*. 2006b). Furthermore, the testate amoebae assemblages from North American and British salt marshes were surprisingly similar, suggesting similar environmental controls on taxon distribution and the potential for creating robust regional transfer functions applicable to fossil assemblages from a variety of locations (Charman *et al*. 2010). Other potential advantages of using testate amoebae as an additional sea-level indicator are that the range of sediments that can yield sea-level information can be extended into supratidal settings (Ooms *et al*. 2011), and that higher precision estimates of palaeo-sea level are possible, especially when combined with foraminifera and diatoms (Gehrels *et al*. 2001).

The only comprehensive salt-marsh surface testate amoebae data come from the aforementioned studies in the UK and North America and from the Scheldt estuary in Belgium (Ooms *et al*. 2011, Ooms *et al.* 2012). To date, the only fossil coastal data sets that have been described are from salt marshes in Maine (USA) and Nova Scotia (Canada; Charman *et al*. 2010) and a range of short and fragmentary sediments in the UK (Roe *et al.* 2002). In order to establish the spatial variability shown by salt-marsh testate amoebae, additional surface data sets from locations across the North Atlantic region are required. In addition to this, largely due to the limited number of studies on salt-marsh testate amoebae, preparatory and analytical techniques vary for this proxy. The time consuming nature of testate amoebae data collection and variable preparation techniques available often causes problems in generating large data sets from both modern and fossil samples (Roe *et al.* 2002, Charman *et al.* 2010). With this in mind, there are three main aims associated with this study: 1. Assess the statistical significance of different specimen counts per sample; 2. Compare and standardise sample preparation techniques; 3. Assess the relationship between salt-marsh surface testate amoebae and surface elevation at two salt marshes located in northwestern Norway.

MATERIALS AND METHODS

Study sites and field sampling

Samples were collected from two salt marshes located on the south coast of Hinnøya, an island in the Vesterålen archipelago in northwestern Norway (Fig. 1). The two salt marshes are Storosen (68°20′50″N, 15°41′26″E) and Svinøyosen (68°20′15″N, 15°33′06″E), which are situated 6 km apart. They share similar tidal characteristics; mean tidal range is 2.6 m during spring tides and 1.2 m during neap tides, mean sea level (MSL) is 1.7 m above chart datum (lowest astronomical tide; LAT) and highest astronomical tide (HAT) is 3.7 m above datum. These tidal data have been extrapolated from the two nearby secondary ports of Lødingen and Kabelvåg. Storosen is the smaller of the two marshes, about 3,500 m^2 in size and located between a small tidal stream and an established copse of trees. Vegetation characteristics were used to define the marsh zonation. The elevation of HAT (2 m above MSL) is characterised by grasses (e.g. *Leymus arenarius* and *Arrhenatherum elatius*) and mixed herbaceous plants. Below this, the high marsh extends from ~ 1.7 m above MSL down to ~ 1.3 m above MSL and is dominated by *Juncus gerardii.* The low marsh zone is dominated by *Blysmus rufus* and also contains *Juncus ranarius* and *Plantago maritima* (Fig. 2) and extends from ~ 1.3 m above MSL down to

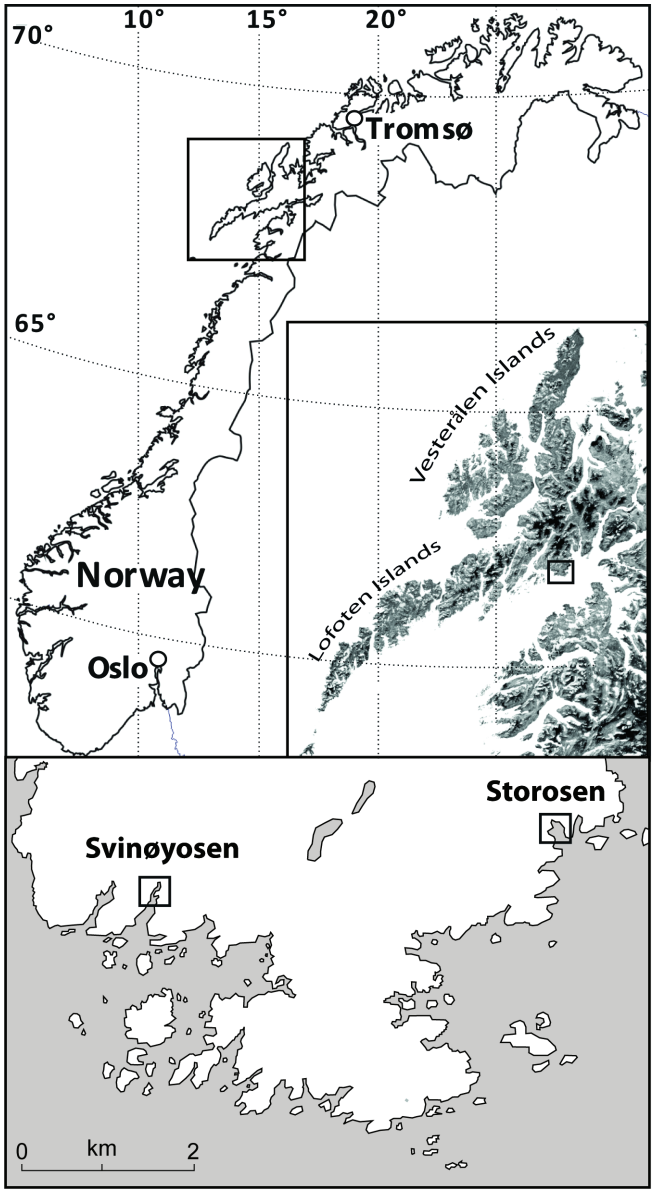

Fig. 1. Location map of the field sites.

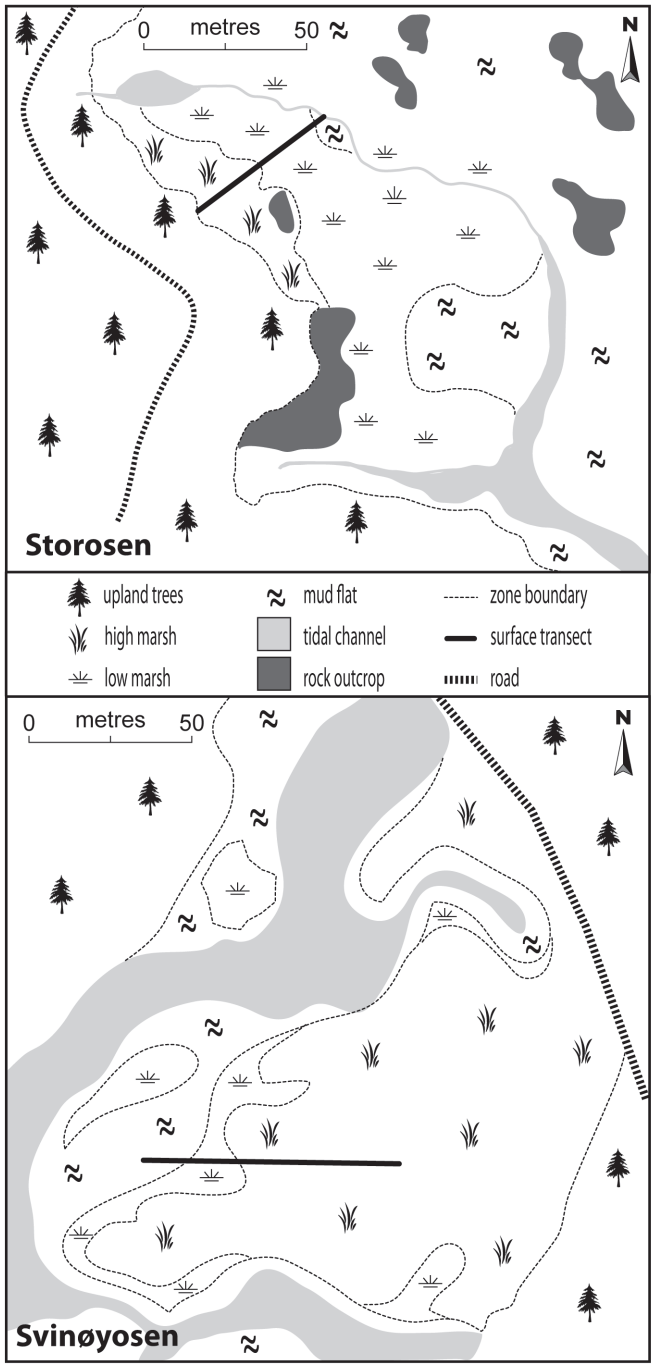

Fig. 2. Schematics of Svinøyosen and Storosen showing vegetation zonation.

~ 1.1 m above MSL. Below these elevations bare mud flats dominate the environment. Svinøyosen is the larger and more developed of the two marshes, about 12,000 m^2 in size. A similar plant zonation can be seen. The HAT elevation is characterised by a diverse flora of mixed grasses and herbs. With decreasing elevation from this zone (~ 1.6 to ~ 1.2 m above MSL) *Juncus gerardii* dominates the environment down until the low marsh zone (~ 1.2 to 0.9 m above MSL) which is occupied by *Juncus ranarius*, *Triglochin maritima* and *Plantago maritima* (Fig. 2).

During the summer of 2010 transects were laid out across the salt marshes at Storosen and Svinøyosen from near HAT, through the high and low marsh zones and onto tidal mud flats. A benchmark was located at each site and related to mean sea level (Norway Geoid 2008), using a Trimble differential global position system (DGPS) RTK base station (± 0.5 cm horizontal error; ± 0.7 cm vertical error). Surface transects were surveyed relative to this benchmark at 1 m intervals using a Trimble 5600 total station (± 0.24 cm vertical error). Surface samples were taken along the transects at regular

height change intervals of approximately 4 cm. A total of 23 surface samples (diameter 80 mm, depth 30 mm) were collected from Storosen, and 18 from Svinøyosen using a circular tin (Pitman) corer.

Testate amoeba analyses

Preparation procedures for salt-marsh testate amoebae are not yet standardised but are commonly based on methods designed for peatland testate amoebae (e.g. Ooms *et al.* 2011), or foraminifera studies (e.g. Riveiros *et al*. 2007). A particular problem for salt-marsh studies is that concentrations of tests are quite low and sediment particles in the same size range as testate amoebae can obscure tests on the slides. Charman *et al*. (2010) adapted a peatland preparation technique using mild alkali and repeat sieving in an attempt to increase the concentration of tests on microscope slides. These efforts helped to reduce counting time. Here we develop and test this process further to improve analytical efficiency. Another approach to reduce analysis time is to reduce the number of individual specimens counted per sample. A count total of 150 has often been suggested for palaeoecological analysis of testate amoebae (Warner 1990, Gehrels *et al*. 2006b), but a lower count total may be sufficient when species diversity is relatively low (Payne and Mitchell 2009). Given that higher count totals are often impossible to achieve, count totals of 100 or even less are sometimes used (Charman *et al*. 2010, Ooms *et al*. 2011). Here we examine the impact of lower counts on the representativeness of assemblages.

The testate amoeba analyses were divided into three parts. First, a subset of samples was analysed to assess the effects of using a lower count total (100 compared with 150 tests) on the assemblage data. Second, a comparison was made between subsets that were subjected to various preparation techniques in an attempt to improve the efficiency and effectiveness of recovery and counting of tests. Finally, a full set of samples was analysed for use in multivariate analysis with environmental data to test the relationship between elevation and taxon assemblages. All taxon identification was performed under a light microscope (400 × magnification) using the classification of Charman *et al*. (2000), supplemented by Charman *et al.* (2002). All surface data refer to counts of dead (unstained) tests in order to help reduce any bias from effects of seasonal blooming. Similar to foraminiferal studies, it is believed that dead assemblages of micro-organisms more closely resemble fossil assemblages and therefore should normally be used in developing modern analogue data (Murray 1982, 2000).

A selection of samples was taken from Storosen in order to assess the effects of different count totals on the representativeness of testate amoebae assemblages used for sea-level reconstruction. Storosen is the younger of the two marshes and shows a simpler flooding regime with less of a freshwater input, therefore samples from here were the suitable choice when investigating the analytical procedures involved with testate amoebae analysis. Samples from elevations between HAT and mean high water springs (MHWS; 1.35 m above MSL) were selected in order to ensure diverse populations of testate amoebae were recovered. Above these elevations testate amoebae populations show less response to tidal inundation (Ooms *et al.* 2012), and below these elevations testate amoebae populations show less diversity (e.g. Charman *et al.* 2010), and would thus be of limited use for investigating analytical procedures. A subsample of 2 cm^3 was taken from the top 1 cm of each surface sample and prepared using procedure A (Table 1). This preparation is similar to that most commonly used in analysis of peat samples (Hendon and Charman 1997, Charman *et al*. 2000). It uses only water-based disaggregation and sieving to minimise damage to tests from any chemical treatment. Assemblage counts were performed on each

Table 1. Details of the three preparation techniques applied to six replicate samples from Storosen.

<table>
<tr><th>A</th><th>B</th><th>C</th></tr>
<tr><td colspan="3">Take 2 cm^3 of sediment from the top 0.5 cm of a surface sample;
stain and store in Rose Bengal for a minimum of 48 hours</td></tr>
<tr><td colspan="3">In a 250 ml glass beaker add two Lycopodium clavatum L. tabs (Stockmarr 1971) and dissolve in
10 ml of [10%] HCl; add the stained sample and dilute immediately with 150 ml deionised (DI) H^2O</td></tr>
<tr><td colspan="3">Warm beaker on a hot plate at 80°C for 1 hour, stirring every 15 minutes to aid disaggregation of
material. Turn off hot plate and leave the sample to cool slowly for a minimum of 12 hours</td></tr>
<tr><td colspan="2">Sieve the sample and retain the fraction > 15 µm < 300</td><td>Wash the sample through
a 15 µm sieve, retaining
the > 15 µm fraction</td></tr>
<tr><td></td><td colspan="2">Transfer the sample back into the beaker using 25 ml
DI H^2O; add 4 ml [5%] KOH and heat immediately
on a hot plate at 80°C for precisely 2 minutes</td></tr>
<tr><td></td><td>Dilute the sample with DI H^2O
and wash through a 15 µm sieve, retaining the
> 15 µm fraction</td><td>Dilute the sample with DI H^2O
and sieve, retaining the
> 15 µm < 212 fraction</td></tr>
<tr><td colspan="3">Transfer the sample into a 30 ml Sterilin tube and centrifuge at 3,000 RPM for 5 minutes</td></tr>
<tr><td colspan="3">Remove the supernatant and mount onto a glass microscope slide; use DI H^2O if
counting straight away or glycerol jelly if the slides are to be stored</td></tr>
</table>

sample until 100 individuals were recorded to species level. The counts were then continued until 150 individuals were identified.

In order to analyse the impacts of applying different preparation techniques, additional 2 cm^3 subsamples from six of the eleven samples were prepared using procedures B and C (Table 1). Both of these procedures include a weak alkali treatment designed to assist in the disaggregation of the samples. Procedure C also reduces the upper particle size of the analysed fraction to 212 µm in an attempt to exclude larger sediment particles whilst retaining the largest testate amoebae. Where samples were prepared using multiple methods a total of 6 cm^3 was taken from the top 1 cm of the surface sample, homogenised, and then split into 2 cm^3 portions in order to remove the possibility of bias related to micro-scale spatial variation in assemblage composition and test concentration. Count totals of 100 individuals were used to compare the different preparation techniques. Finally, to provide a full training set of salt-marsh testate amoebae all 18 surface samples from Svinøyosen were subsampled (2 cm^3) and prepared using procedure C. Count totals for these samples were 100 tests.

Data analysis

The assemblages based on different count totals (100 and 150), were directly compared to determine whether the count totals significantly altered assemblage compositions. Bray-Curtis Index tests (BCI; Bray and Curtis 1957) were used to compare dissimilarities between the assemblages. These tests take into account the presence and absence of species encountered, as well as abundance information.

Statistical comparisons of assemblages often make an assumption that the full ecological populations are known. However, assemblages that have been constructed with a limited count total may fail to take into account certain rare species if they are not seen. Occurring in very low numbers, these rare species still legitimately belong to the ecological population of a sample. With this in mind, the assemblages used to investigate different preparation techniques were compared using Chao's Sørensen abundance-based similarity estimator (Chao *et al*. 2005). This test makes an estimate of the unseen shared species based on the frequencies of observed rare, shared species, correcting for the effects of unseen species. Simulated estimations from known plant assemblages containing unseen species have shown the test infers the presence of unseen species well and reduces bias associated with compared but unadjusted data sets (Chao *et al.* 2006).

The EstimateS software package (v. 7.5; Colwell 2005) was used to apply the different indices tests to the datasets. The software program C^2 (v. 1.4.3; Juggins 2003) was used to plot surface assemblage data against elevation and apply a weighted averaging regression model to the data set. Constrained cluster analysis was performed using CONISS (Grim 1987) within the software program Tilia (v. 1.7.16; Grim 1992) and detrended canonical correspondence analysis (DCCA) was run using Canoco (v. 4.56; Jongman *et al*. 1995).

RESULTS

Effects of different count totals

The eleven pairs of assemblages produced from Storosen using different count totals are presented in Fig. 3. Dominant species (such as *Centropyxis cassis* type and *Cyclopyxis arcelloides* type) are consistently represented in similar percentages by the two count totals. Assemblage differences are mainly driven by taxa which are encountered in very low numbers. Table 2 presents some quantitative characteristics of these

Table 2. Assemblage characteristics for the count total analyses.

Elevation m above MSL	Count total	Total taxa	No. taxa > 2% / rare taxa	No. tests per cm^3 ($\times 10^2$)	BCI
1.753	100	14	7 / 7	251	
	150	15	7 / 8	245	0.07
1.707	100	21	10 / 11	282	
	150	22	9 / 13	228	0.09
1.670	100	15	11 / 4	211	
	150	17	10 / 7	202	0.06
1.612	100	14	10 / 4	211	
	150	15	10 / 5	221	0.09
1.571	100	11	6 / 5	1689	
	150	11	7 / 4	1394	0.04
1.514	100	12	8 / 4	216	
	150	12	8 / 4	251	0.04
1.492	100	11	6 / 5	282	
	150	12	7 / 5	300	0.04
1.468	100	14	7 / 7	96	
	150	15	7 / 8	107	0.05
1.418	100	8	5 / 3	286	
	150	8	5 / 3	320	0.07
1.392	100	11	6 / 5	86	
	150	14	7 / 7	36	0.06
1.360	100	10	7 / 3	24	
	150	11	7 / 4	25	0.08

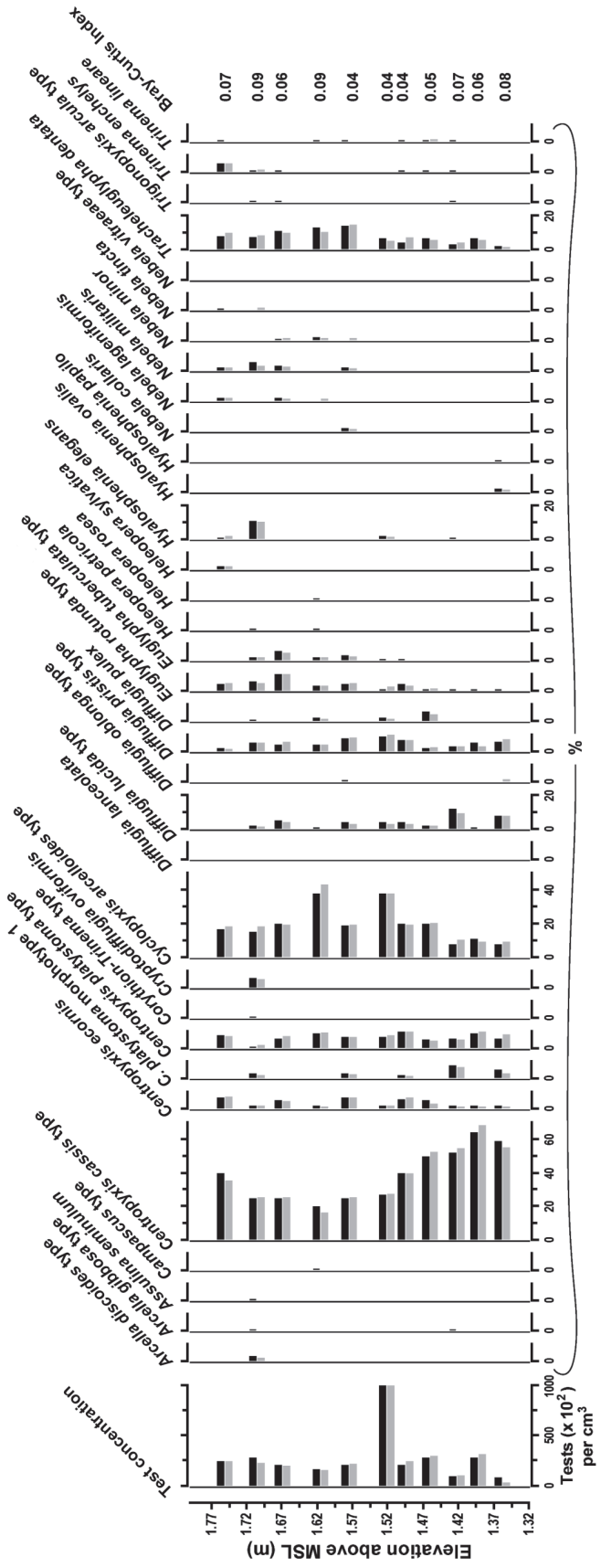

Fig. 3. Testate amoebae assemblage data from eleven samples after applying a count total of 100 (black) and 150 (grey).

assemblages and includes the results from the BCI comparative statistics. On average, by continuing the count total to 150 individuals, rather than stopping at 100, only a single additional taxon was encountered at low abundance. By dividing the assemblages into rare (those making up 2% or less of the assemblage) and non-rare (> 2%) species it is evident that the total number of non-rare taxa remains more or less consistent. The number of non-rare taxa never varies by more than 2 when using the different totals. Variations in the total number of taxa are largely driven by the presence of rare taxa, which can often be represented by a single occurrence in a sample.

There is a clear tendency for a reduction in the taxon diversity with decreasing elevation. The lower samples typically contain between 8 and 12 taxa, regardless of count totals, whereas the higher elevation samples contain between 11 and 22 taxa. The lowest concentration of tests is found in the lowest sample at 1.36 m above mean sea level (MSL) where there are approximately 2,400–2,500 tests cm^{-3}. The samples from higher elevations most commonly contain between 20,000 and 30,000 tests cm^{-3}, although numbers are sometimes much higher and highly variable.

A high BCI score, close to 1, indicates that pairs of assemblages are virtually entirely different in terms of the taxa present and their relative abundances within the assemblages (e.g. Magurran 2004). All eleven pairs of assemblages have a BCI score of less than 0.1 suggesting that assemblage compositions are nearly identical, regardless of whether count totals of 100 or 150 are used. Again, minor dissimilarities seen between the assemblages are driven by the presence or absence of rare species.

Effects of different preparation techniques

Assemblage compositions and a summary of assemblage characteristics from the six samples where three different treatments were used are presented in Fig. 4 and Table 3 respectively. Preparation method A, which contained no chemical disaggregation procedures, yielded the highest number of taxa per sample. On average, method A produced 2 and 2.33 more species per sample compared to preparations B and C, respectively. On two occasions preparation B yielded the highest number of taxa, and only once did method C produce the highest number of taxa. As a rule the different preparation techniques produced similar ratios of non-rare taxa to rare taxa, with very few exceptions. The lowest elevation (1.39 m above MSL) is again associated with

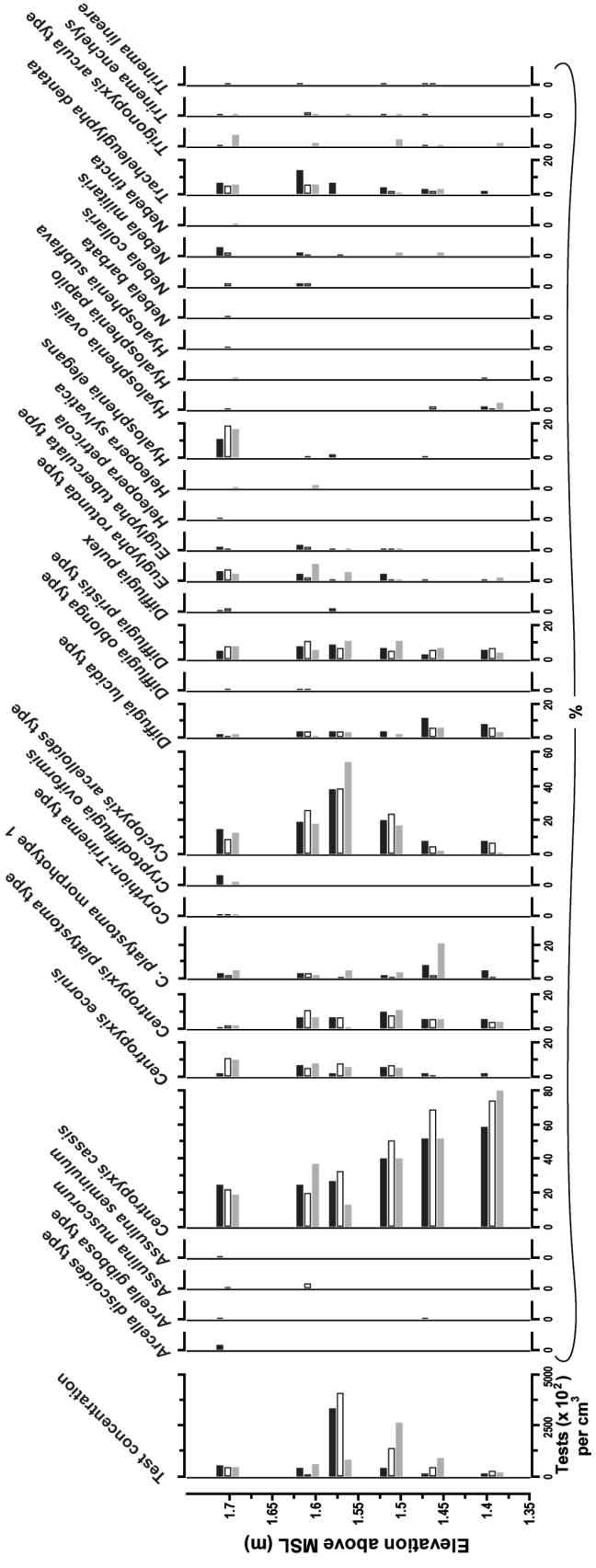

Fig. 4. Testate amoebae assemblage characteristics from six samples following preparation methods A (black), B (white) and C (grey).

the lowest number of taxa encountered. The largest number of taxa is found in the highest elevation sample. In terms of test concentrations, method C produced the highest number of tests per cm^3 of sediment in three out of the six cases. In three cases method A yielded the lowest concentrations of tests. As replicate samples should in theory contain a similar concentration of tests per unit volume, regardless of preparation technique, a low concentration may indicate that some individuals are being 'missed' during the counting process, either by being hidden in debris on the slide, lost during sieving or damaged and dissolved by the treatment.

As was seen earlier, the presence or absence of rare species can influence the characteristics of an assemblage significantly. Incomplete inventories may ignore species with low abundances (e.g. Longino *et al*. 2002, Pfeiffer and Mezger 2012) resulting in a catalogue of unseen species, which remain unaccounted for. When comparing two assemblages with sub-maximal count totals, such as in this case, it is important to adjust for these unseen species. Chao's Sørensen abundance-based similarity estimator makes a probability based estimate of the rare species that are shared, yet not necessarily seen, in both assemblages (Chao *et al*. 2005). The similarity index is then applied following this correction and ranges between 0 (perfectly dissimilar) and 1 (perfectly similar). All except one of the comparisons show an index score of greater than 0.9 and thirteen of the total eighteen comparisons show an index score of greater than 0.95. When unseen species are accounted for it is therefore apparent that the assemblages produced from the different preparation techniques are very similar.

Assemblage relationship with elevation

The results from the 18 surface samples taken from Svinøyosen were combined with the eleven samples used in the analysis on count totals from Storosen and are presented in Fig. 5. All assemblages were derived from count totals of 100 for consistency. Samples below 1.36 m above MSL were sparse in testate amoebae and where a count total of 100 was not feasible a reduced count total of 50 was applied. Count totals for the two lowest samples (0.94 and 0.91 m above MSL) only reached 37 and 7 respectively due to the scarcity of testate amoebae in the samples. The highest number of taxa was consistently encountered at higher elevations, above mean high water springs. Samples with the fewest taxa were located at the lowest elevations and the lowest sample contained only one taxon, *Cen-*

Table 3. Assemblage characteristics for the preparation analyses.

Elevation m above MSL	Preparation method	Total taxa	No. taxa > 2% / rare taxa	No. tests per cm^3 ($\times 10^2$)	Chaos estimate	95% confidence intervals
1.707	A	21	10/ 11	282	A : B = 0.91	± 0.10
	B	21	7 / 14	238	B : C = 0.88	± 0.15
	C	17	9 / 8	235	A : C = 0.93	± 0.12
1.612	A	14	10 / 4	211	A : B = 0.97	± 0.05
	B	16	9 / 7	60	B : C = 0.94	± 0.09
	C	12	7 / 5	300	A : C = 0.96	± 0.10
1.571	A	11	6 / 5	1689	A : B = 0.92	± 0.27
	B	8	6 / 2	2064	B : C = 0.98	± 0.11
	C	10	7 / 3	422	A : C = 0.98	± 0.15
1.514	A	12	8 / 4	216	A : B = 1.00	± 0.05
	B	9	5 / 4	714	B : C = 1.00	± 0.10
	C	13	7 / 6	1327	A : C = 0.98	± 0.09
1.468	A	14	7 / 7	96	A : B = 0.96	± 0.08
	B	10	5 / 5	241	B : C = 0.97	± 0.10
	C	9	6 / 3	464	A : C = 0.96	± 0.12
1.392	A	11	6 / 5	86	A : B = 1.00	± 0.06
	B	7	5 / 2	159	B : C = 0.99	± 0.10
	C	8	5 / 3	104	A : C = 0.96	± 0.18

tropyxis platystoma morphotype 1. No testate amoebae were encountered below this elevation (0.91 m above MSL) and low concentrations of tests were encountered below 1.42 m above MSL.

We have distinguished the morphotype *Centropyxis platystoma* morphotype 1 on the basis of similarities with the salt-marsh testate amoebae taxa named 'Type C' in Charman *et al.* (2002). Our specimens clearly belong to the *Centropyxis cassis-platystoma* complex (Ferry Siemensma, pers. comm. 2012), yet are sufficiently distinguishable from typical *Centropyxis platystoma* type (Charman *et al.* 2000) due to their shape and size (Fig. 6). The taxon does not meet the criterion of twice length by breadth usually used to determine *C. platystoma* type in sediments (Charman *et al.* 2000) and is consistently found to be much smaller at 20–40 µm in length. The aperture face is approximately at 90° (or often shallower) to the long axis of the test which is composed of small, agglutinated particles, as is common for *Centropyxis* and *Difflugia* types. In our study, *Centropyxis platystoma* morphotype 1 occurs in samples of low elevation in comparison to *C. platystoma* type which is most abundant at higher elevations, above mean high water springs. We have therefore made a tentative separation of this taxon here as it may prove to be a useful sea-level indicator.

Constrained cluster analysis was used to help partition the modern testate amoebae data into zones of similar faunal compositions in relation to elevation. The analysis identified three main zones of assemblages. The highest zone (HIN 1) is found between 1.5 and 1.75 m above MSL and is characterised by a high diversity of taxa (up to 22) and a high concentration of tests (> 20,000 cm^{-3}). *Centropyxis cassis* type occurs as a dominant taxon and typically makes up half of the assemblages. *Centropyxis ecornis*, *Centropyxis platystoma* type, *Cyclopyxis arcelloides* type and *Trachleuglypha dentata* are also characteristic taxa and are all present in percentages greater than 10%. Numerous other taxa occur in lower numbers including *Euglypha* spp., *Nebela* spp. and *Trinema* spp. Zone 2 (HIN 2) extends from 1.5 to 1.15 m above MSL. Here, *Centro-*

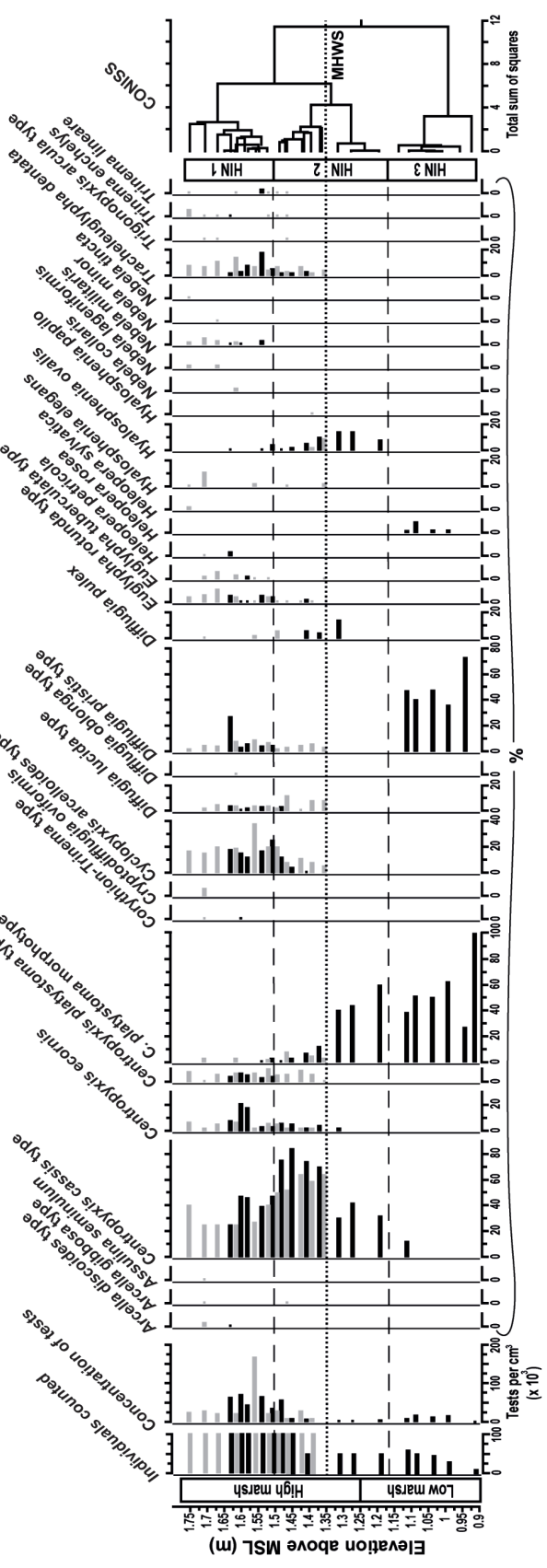

Fig. 5. Combined surface data from Svinøyosen (black) and Storosen (grey). Constrained cluster analysis is used to identify sample zonation relative to elevation above MSL (HIN 1, 2 and 3). The level of mean high water springs is shown (dotted line).

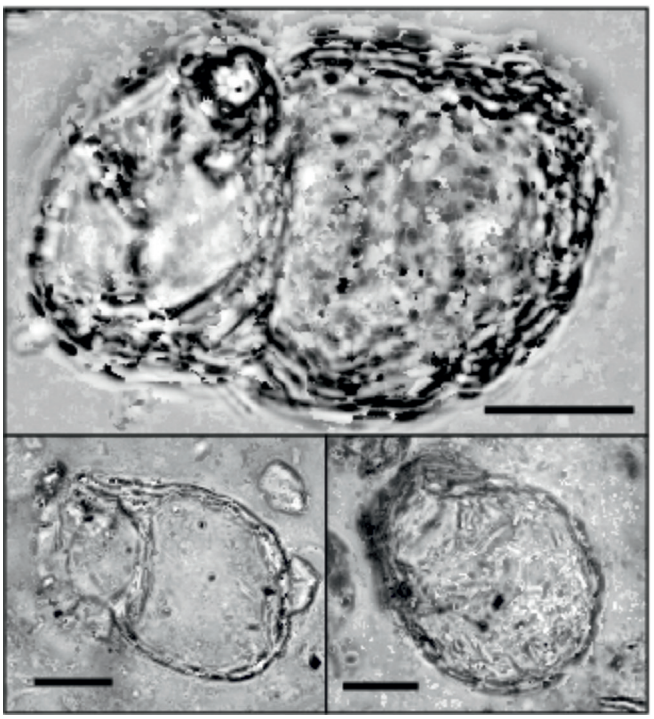

Fig. 6. Micrograph images of *Centropyxis platystoma* morphotype 1 (described in text). Scale bars: 10 µm.

pyxis cassis type makes up a higher percentage of the assemblages than before (~ 75%). There are fewer rare species such as *Euglypha* spp., *Nebela* spp. and *Trinema* spp. although *Hyalosphenia ovalis* often occurs in this group. *Tracheleuglypha dentata* is still present in the higher samples of group 2 but is less abundant. The proportion of *Centropyxis platystoma* morphotype 1 increases with the lower samples of the group at the expense of other *Cyclopyxis* and *Centropyxis* taxa. Finally the lowest elevation samples (HIN 3) typically contain few species (one to three). *Centropyxis cassis* type is no longer present and two species dominate the assemblages, *Centropyxis platystoma* morphotype 1 and *Difflugia pristis* type.

DISCUSSION

Determining a count total

Despite there being little research performed on the analysis of testate amoebae from salt marshes, results from peatland studies have given some direction on

the preparation and analysis of this group of micro-organisms. There is evidence that count totals beyond 60 individuals will not necessarily reduce standard errors of counting and that a total of 100 tests is sufficient for redundancy analysis and transfer function development and application (Payne and Mitchell 2009). Previous studies also indicate that observed species diversity increases little, if at all, once a total of 100 individuals have been counted (Woodland *et al*. 1998, Mitchell *et al*. 2000). From the high BCI scores seen across all eleven comparisons, the data from Norway suggest there is little statistical difference seen between assemblages composed of 100 or 150 tests. With this in mind, the time saved from the lower count total may permit the inclusion of more samples in a transfer function training set or a higher resolution set of fossil counts to be produced. Unsurprisingly, counting higher total numbers reveals additional rare taxa, but the percentage counts of these are so low that they are unlikely to affect environmental interpretation of the assemblage. Studies concerned with generating full taxon lists of testate amoebae present should use a minimum count total of 150 as shown here and by Payne and Mitchell (2009), but palaeoenvironmental interpretation can be successfully based on count totals of 100. Indeed, models including some samples based on lower counts have been published and show that salt-marsh testate amoebae have strong predictive power (Charman *et al*. 2010), although a minimum count of 100 tests is still preferred (e.g. Ooms *et al*. 2011).

Salt-marsh testate amoebae preparation

Almost all salt-marsh testate amoebae studies have employed the use of a water-based soaking method in order to prepare surface samples for analysis (Charman *et al*. 1998, 2002; Gehrels *et al*. 2001, 2006b; Ooms *et al*. 2011). Testate amoebae prepared using more destructive methods, such as those used in palynology, can lead to loss of tests and assemblage bias, although total concentrations may be higher due to more effective sample disaggregation (Hendon and Charman 1997, Payne *et al*. 2012). A water-based technique will ensure the survival of individual tests, but may be insufficient to disaggregate fibrous salt-marsh peat. This leads to three problems. First, if tests remain attached to conglomerate organic particles they may not pass through the larger sieve. Second, they could be concealed by debris and be missed. Third, due to crowding of clumped sediment particles on the microscope slide, the time required for counting will increase significantly.

Charman *et al.* (2010) used a weak alkali treatment of 5% KOH in the preparation of testate amoebae that did not lead to the significant loss of tests. Here, preparation A is largely based on the water soaking method for peat samples outlined in Charman *et al.* (2000). Preparation B is largely based on the techniques used in Charman *et al*. (2010) including a mild alkali to aid disaggregation, and preparation C is a development of this method using a smaller upper sieve size to remove larger non-testate particles. Apparently low concentrations of testate amoebae from technique A suggest that not all individuals were counted, possibly because of slides being crowded with organic conglomerate particles and a dense matrix of small inorganic particles. The average length of time required to reach a count total of 100 was approximately 13 hours, and the longest time spent on one sample was 22 hours (Fig. 7). In comparison, the samples prepared using methods B and C took a lot less time to count, the shortest time taken to reach 100 tests being 15 minutes (Fig. 7). The high Chao's Sørensen abundance-based similarity index-scores indicate that assemblages produced using the different preparation procedures are actually very similar. Minor dissimi-

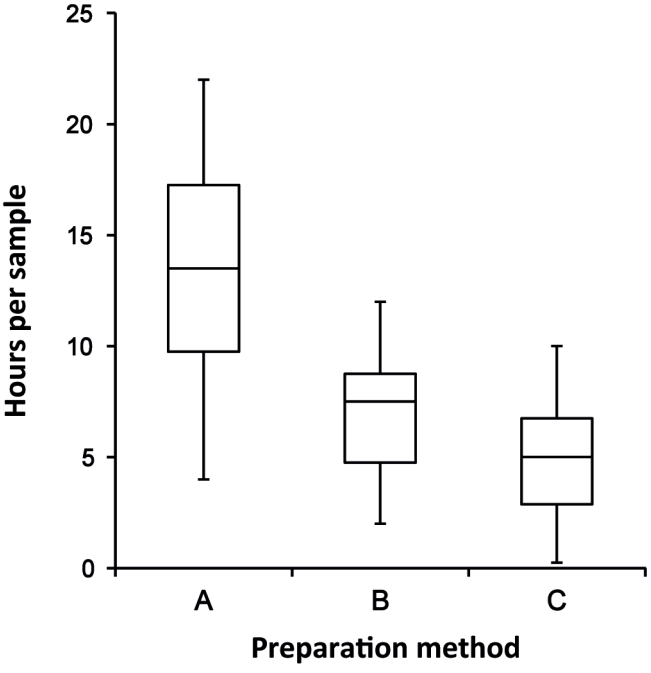

Fig. 7. Box and whisker plots showing maximum, minimum and median time spent counting assemblages of 100 individuals following different preparation procedures.

larities between the populations are likely to have been driven by the effects of unseen rare species. So, for the purposes of palaeoenvironmental research, where a full complement of taxa is not required, there are time gains to be made from using a weak alkali treatment with potentially very little loss of information from the destruction of tests.

Preparation C was devised for two reasons. First, sieving prior to the alkali treatment in method B may have led to the loss of tests that were encompassed within organic conglomerate particles greater than 300 µm in size. Second, throughout the entire counting process no testate amoebae greater than 160 µm in size were encountered. Therefore in order to reduce crowding on microscope slides further, a smaller upper mesh sieve of 212 µm was used following the alkali disaggregation. Samples prepared using this method, as a whole, had the shortest count times (Fig. 7). However, the use of a sieve smaller than 300 µm is only advised if it is known that there will be no larger taxa present in the samples, checks of the residue retained in the sieve is advisable, yet this may negate time saved using this method.

Norwegian salt-marsh testate amoebae as sea-level indicators

The surface data presented here from Storosen and Svinøyosen show some remarkable similarities with published patterns of salt-marsh testate amoebae from the UK (Gehrels *et al*. 2001, Charman *et al.* 2002) and from North America (Gehrels *et al*. 2006b). For all three locations most taxa exhibit similar microhabitat preferences across the marsh. *Euglypha* species are most commonly found at the higher end of the transects, alongside *Tracheleuglypha dentata*, *Trinema* spp., and *Assulina* spp. *Centropyxis cassis* type is one of the most common taxa and is consistently located throughout the high marsh, but is never found in the lower parts of the marsh. A similar distribution is apparent for *Cyclopyxis arcelloides* type. *Difflugia pristis* type is largely associated with the low marsh and is clearly more tolerant of higher salinities and longer submergence periods than many other forms of testate amoebae taxa. The constrained cluster analysis for the Norwegian data set shows a grouping of samples near the highest astronomical tide level, a second group occurring around the level of mean high water springs and a final, lowest group existing between mean high water neaps and mean high water springs. It appears that testate amoebae are unable to survive at elevations below the level of mean high water neaps, or perhaps unable to compete with other organisms such as foraminifera. The lowest samples from North America and the UK always show more diverse populations of testate amoebae in comparison to Norway where only two taxa are present. This may be due to the more northern location of the Norwegian sites, which could decrease taxon diversity similar to that found for the north-south gradient in the Arctic (Beyens *et al*. 1986). The zonation shown by salt-marsh testate amoebae from locations across the North Atlantic gives strong evidence of their potential as sea-level indicators.

To provide an example of the predictive power of testate amoebae the Norwegian surface data set was used to build a transfer function in C[2] (Juggins 2003). The training set of 29 samples had a DCCA gradient length of > 2 standard deviations on axis 1, suggesting a unimodal taxon response to the environmental variable, in this case, elevation (ter Braak and Prentice 1988, Birks 1995). Weighted averaging regression was therefore a suitable model to calculate taxon-specific elevational optima and the tolerances (Fig. 8). The majority

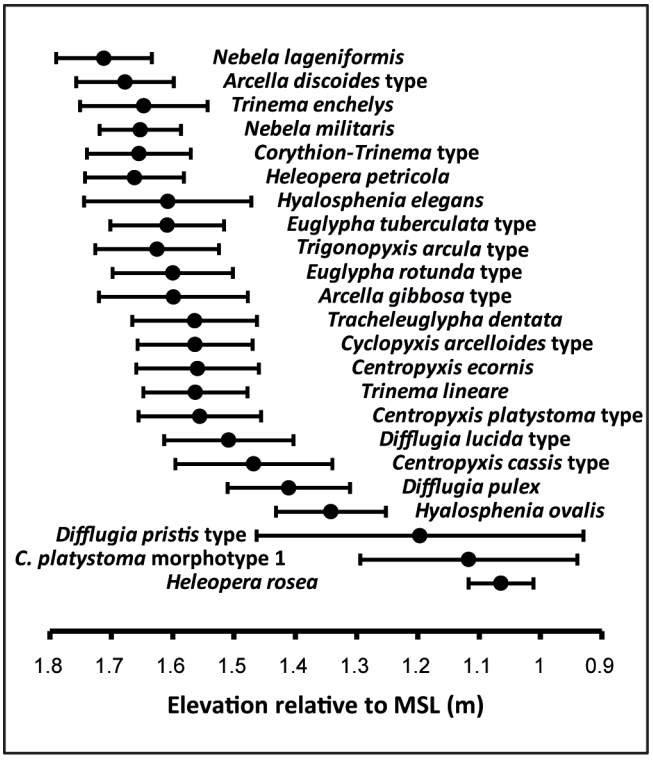

Fig. 8. Optima and tolerance ranges for taxa using cross-correlated, weighted averaging regression analysis. Species occurring in only one sample are omitted.

of testate amoebae taxa found across the salt marshes occur at elevations above mean high water springs (1.35 m above MSL). Below this level only a few, more saline-tolerant taxa exist, significantly *Difflugia pristis* type and *Centropyxis platystoma* morphotype 1. Another important low marsh taxa is *Difflugia pulex* which was also noted by Gehrels *et al*. (2006b) as a useful indicating species. Taxa that occur in abundance and also in defined elevational ranges (and are therefore useful sea-level indicators) include *Centropyxis cassis* type*, Centropyxis ecornis, Cyclopyxis arcelloides* type and *Tracheleuglypha dentata*. Rare taxa that occur in tightly constrained elevational ranges, principally the highest elevations, include *Euglypha* spp. and *Nebela* spp. Despite occurring in low numbers these semi-terrestrial taxa may still be useful in establishing past sea levels.

To test the example model, predicted sample elevations from the transfer function were plotted against the 29 observed sample elevations (Fig. 9). In almost all cases observed sample elevations are accurately reconstructed by the transfer function. The R^2 value for the cross-correlated model is 0.86, showing good agreement between the observed and predicted elevations. By using testate amoebae alone and a weighted averaging (classical deshrinking), cross-correlated transfer function, marsh elevations can be predicted to within a precision of ± 0.09 m (RMSEP: 0.091). By including another sea-level indicator such as foraminifera in a predictive model, it is likely that this accuracy can be improved upon even further (Gehrels *et al*. 2001).

To conclude, we have shown that, for the purpose of palaeoenvironmental studies using salt-marsh testate amoebae, it can be useful to adapt existing analytical techniques to increase analytical efficiency. With reference to ours aims, we therefore suggest that: 1. By using a lower count total of 100, rather than 150, for sample populations, time gains can be made during the analyses stage. This development is advisable for palaeoenvironmental reconstruction where time can be spent valuably elsewhere (e.g. by increasing the number of samples analysed), however, it is not advised for studies concerned with evaluating full ecological populations of taxa; 2. By including a mild alkali as a chemical disaggregant, testate amoebae can be concentrated on microscope slides with little or no loss of taxon information; 3. Testate amoebae from two salt-marshes in north-western Norway show sufficient zonation relative to elevation that they can potentially reconstruct sea level to within ± 0.09 m.

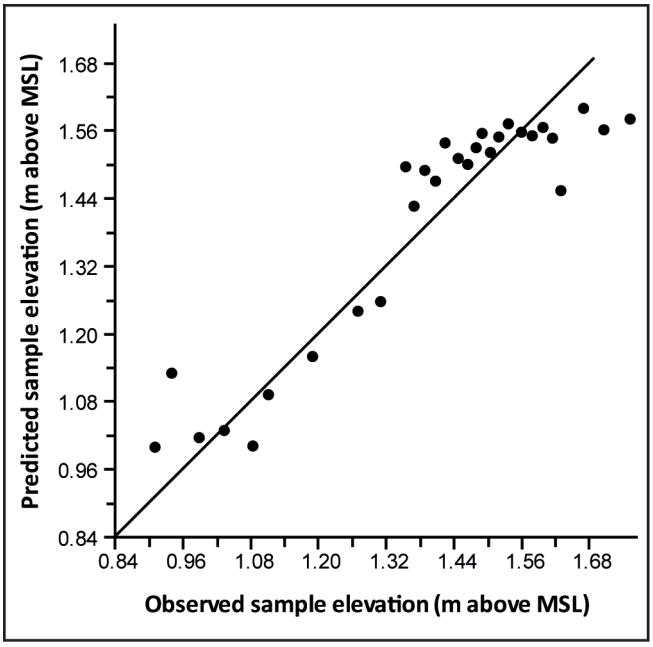

Fig. 9. Predicted versus observed sample elevations.

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