

1 **Reconstruction of recent sea-level change using testate amoebae**

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12

13 **Abstract**

14 Proxy-based sea-level reconstructions place the instrumentally observed rates of recent sea-
15 level rise in a longer term context by providing data that extend the instrumental sea-level
16 record into past centuries. This paper presents the first sea-level reconstructions based on
17 analyses of testate amoebae, to test their capability to improve the precision of past sea-level
18 reconstructions. We used sample preparation methods commonly used in peatland studies,
19 but applied a modified sample preparation based on a weak alkali treatment to improve the
20 efficiency of the counting process. We present two reconstructions for the past 100 years
21 from sites in Maine (USA) and Nova Scotia (Canada) based on short cores from salt
22 marshes and modern training data from North America and the United Kingdom. These are
23 compared with tide-gauge records and reconstructions based on foraminifera from the same
24 cores. The reconstruction from the Chezzetcook marsh (Nova Scotia) shows good
25 agreement with both the tide-gauge and the foraminifera-based reconstruction and is
26 insensitive to the modern training set and underlying transfer function model. The Wells
27 (Maine) reconstruction is also consistent with the tide-gauge record and foraminifera-based
28 reconstruction, but shows greater variability depending on methodology applied. The
29 precision of testate amoebae-based reconstructions is higher than those for foraminifera, but
30 further work on surface samples is needed to improve the accuracy of reconstructed values.
31 The UK data perform well in predicting known elevations of North American surface samples,
32 and sea-level reconstructions based on the UK transfer function are very similar to those
33 based on the North American data, suggesting the methodology is robust across large
34 geographical areas. We conclude that testate amoebae have the potential to provide robust,
35 higher precision sea-level reconstructions for the past few centuries if modern transfer
36 functions are improved and core sites are located within the main zone of testate amoebae
37 occurrence on the saltmarsh.

38

39

40 **1. Introduction**

41 Sea-level change is one of the most important aspects of future global change and
42 understanding the causes, rates and geographical and temporal variability of sea level is
43 crucial to improving predictions of 21st century sea-level rise. Measurements and
44 reconstructions of past sea level play an important part in improving understanding of sea-
45 level change (Bindoff et al., 2007). Tide-gauge records and satellite data provide high
46 temporal resolution data, but the temporal and spatial coverage of these records is limited.
47 Reconstructions of sea-level change over the past few millennia are therefore critical to
48 understanding the dynamics of sea-level change and there are a growing number of
49 reconstructions for locations around the world (e.g. Varekamp et al., 1992; van de Plassche
50 et al., 1998; Donnelly et al., 2004; Gehrels et al., 2002; 2005; 2008). Recent research has
51 used high temporal resolution records to show that current rates of sea-level rise are two to
52 three times faster than longer terms rates over previous centuries (Gehrels et al., 2005).
53 Whilst this longer term context for individual locations is important, it does not necessarily
54 help test hypotheses concerning the causes and sources of the recent acceleration in sea-
55 level rise, unless an appropriate spatial and temporal network of reconstructions exists.
56 Adequate spatial and temporal coverage of sea-level change over the last few hundred years
57 can only be provided by palaeoenvironmental records, due to the spatial bias in tide gauge
58 records to the northern hemisphere.

59 The most detailed and precise late Holocene sea-level reconstructions are based on
60 the use of salt-marsh foraminifera as sea-level indicators. Statistical analyses indicate that in
61 most locations the precision of sea-level positions reconstructed from foraminifera is on the
62 order of ± 20 cm (Gehrels, 2000; Edwards and Horton, 2000; Horton et al., 1999a; 1999b)
63 while only along microtidal coastlines, such as the Atlantic coast of Nova Scotia, Canada, the
64 precision is better than ± 10 cm (Gehrels et al., 2005). In most mid-latitude North Atlantic
65 locations the rise of sea level in the past century has been around 10-20 cm (Holgate, 2007;
66 Woodworth et al., 2008). If an attempt is made to detect the onset of this recent rapid rise it is
67 therefore desirable to use sea-level indicators that have higher precision than those offered

68 by foraminifera. Diatoms improve precision in some contexts but testate amoebae appear to
69 have particular potential as high-precision indicators in saltmarsh sediments with tightly
70 constrained zonation in relation to elevation in the upper marsh and supra-tidal zones
71 (Charman et al., 1998; Gehrels et al, 2001; Charman et al., 2002). However, apart from an
72 initial assessment of samples from a range of sedimentary contexts (Roe et al., 2002) there
73 have been no attempts to use testate amoebae to develop and test high resolution records of
74 sea-level change.

75 This paper reports the results of testate amoebae analysis on two saltmarsh cores
76 from the Atlantic coast of North America to demonstrate the use of testate amoebae as high-
77 precision sea-level indicators capable of detecting fluctuations of the order of ± 10 cm over
78 multi-decadal to centennial timescales. Our reconstructions are assessed against nearby
79 tide-gauge data and published sea-level reconstructions based on foraminifera. We also
80 suggest an improved preparation procedure that overcomes some of the difficulties
81 associated with counting low concentrations of testate amoebae in organic silty sediments
82 that are typical of high marsh settings (Roe et al., 2002).

83

84 **Testate amoebae as sea-level indicators**

85 Testate amoebae (or ‘thecamoebians’) are a group of test-forming protozoa some of which
86 are related to foraminifera. They are known to be good indicators of hydrological conditions
87 in peatlands (*e.g.*, Tolonen, 1986; Charman et al., 2006). Testate amoebae have also been
88 reported in salt-marsh environments (*e.g.*, Scott et al., 1991; 1995a) in samples prepared for
89 foraminiferal analyses, but only in low numbers and low diversity in the size fraction 63-500
90 μm . We have collected testate amoebae from surface sediments in salt marshes in the UK
91 (Charman et al., 1998; 2000a; 2002; Gehrels et al., 2001; Roe et al., 2002) and along the
92 eastern seaboard of North America (Gehrels et al., 2006). In these investigations we have
93 applied techniques commonly used in peatland studies (Hendon and Charman, 1997) and
94 examined the fraction between 15 and 63 μm in addition to the 63-500 μm fraction. Our
95 results indicate that small testate amoebae (<63 μm) are much more abundant and diverse

96 in salt-marsh sediments than the larger testate amoebae (>63 μm). The vertical distribution
97 of testate amoebae is distinctly zoned on the surface of salt marshes, and shows a stronger
98 zonation than that of diatoms and foraminifera in the highest levels of marshes (Gehrels et
99 al., 2001). Testate amoebae can be found as low as the level of mean high spring tide but
100 their vertical extent reaches upward into areas well above the highest limit of foraminifera.
101 Based on quantitative analyses of modern datasets we have concluded that testate amoebae
102 may be capable of resolving sea-level positions with a precision perhaps as low as ± 4 cm in
103 microtidal sites, provided that fossil testate amoebae are present and well preserved in
104 subsurface sediments (Gehrels et al., 2006). This study represents the first systematic
105 attempt to use fossil testate amoebae to reconstruct recent sea-level change.

106

107 **Site descriptions and previous work**

108 We sampled cores from two previously studied sites in Maine and Nova Scotia (Figure 1).
109 The Webhannet marsh in Wells, Maine (43°18'N, 70°34'W) is a backbarrier marsh along the
110 Wells embayment in southwestern Maine (USA). The marsh is connected to the Gulf of
111 Maine by a flood-dominated tidal inlet and the mean tidal range in the marsh is 2.64 m
112 (Gehrels, 1994). The sea-level history has previously been determined from salt-marsh plant
113 fossils (Belknap et al., 1989) and foraminifera (Kelley et al., 1995; Gehrels et al., 1996; 2002).
114 The core selected for the present study is core FS-1 from the high marsh zone where
115 *Spartina patens* is the dominant plant cover. This core was first analysed for foraminifera by
116 Gehrels et al. (2002) and formed the basis for a detailed sea-level chronology spanning the
117 past 1200 years. The sea-level curve for Wells compared well with a curve produced for
118 Machiasport in eastern Maine and is therefore interpreted to be representative for the Gulf of
119 Maine. In the past 100-200 years the Gulf of Maine has experienced 0.3-0.4 m of relative
120 sea-level rise, the fastest rise in the past millennium. Gehrels et al. (2002) suggested that
121 sea-level rise may have been slow in the middle of the 19th century, but the precision of their
122 reconstruction precluded a clear distinction between pre- and post-industrial rates of sea-
123 level rise.

124 The Chezzetcook Inlet is located 45 km ENE of Halifax, Nova Scotia (Canada) and is
125 micro- to mesotidal with a tidal range of 2.14 m at the mouth of the inlet. Tidal range is
126 reduced to 1.86 m at the West Head location where core CZ-25 is located in the high marsh
127 zone dominated by *Spartina patens* (Gehrels et al., 2005). Chezzetcook Inlet has been the
128 site of previous Holocene sea-level studies, including those by Scott (1977), Scott et al.
129 (1987; 1995b) and Gehrels et al. (2004; 2005). Gehrels et al. (2005) established a high-
130 resolution sea-level reconstruction for the last 1000 years based on foraminifera and showed
131 a doubling of the rate of sea-level rise, from 1.6 mm yr⁻¹ to 3.2 mm yr⁻¹, shortly after c. AD
132 1900.

133

134 **Laboratory methods**

135 Standard preparations for testate amoebae samples are water-based sieving to separate the
136 15-300 µm fraction for counting at 100-1000x magnification under a light microscope
137 (Charman et al., 2000b). However, in fossil and some surface samples, this process retains a
138 large amount of silt-sized organic remains that can obscure the tests, rendering samples
139 difficult or impossible to count to totals that are appropriate for statistical analysis. Previous
140 assessments on terrestrial peat have shown that typical pollen preparation processes and
141 other chemical treatments damage or destroy tests and so are not generally used. However,
142 low strength alkali treatment prior to sieving can remove organic aggregates. Although there
143 is some alteration of structure, tests are not destroyed and can still be identified (Hendon and
144 Charman, 1997). We used a modified procedure of Hendon and Charman (1997), including
145 pre-treatment with 5% KOH to eliminate unwanted detritus. We compared counts from eight
146 test samples from the Wells core, with and without KOH treatment to assess the effects on
147 test numbers and concentrations. Following this preliminary test, we applied the same
148 preparation process to all fossil samples.

149 Samples of 0.2-0.5 ml from 1cm thick slices were collected from the cores and
150 weighed. One tablet of *Lycopodium clavatum* L. was added as an exotic marker (Stockmarr,
151 1971). Samples were boiled in 100 ml distilled water for 5-10 minutes, stirred occasionally to

152 aid disaggregation and left soaking overnight. The next day, samples were sieved and the
153 fraction of 15-300 μm was retained for analysis. A 4 ml solution of 5% KOH was added and
154 samples were warmed at approximately 80°C for 1-2 minutes and sieved again at 15 μm to
155 remove more fine detritus. Finally, samples were mounted in glycerol or water onto a
156 microscope slide with a 22x40 mm cover slip sealed with nail varnish. In the initial test of
157 KOH treatment, testate amoebae were counted until 150 *Lycopodium* spores had been
158 counted. In the full counts of fossil samples, at least 50 tests were counted. The time-
159 consuming nature of the counting procedure precluded the counting of more specimens, but
160 the number of 50 individuals is sufficient in the absence of rare taxa (those that occur in very
161 low abundances of perhaps <5%; Patterson and Fishbein, 1991). Data from some samples
162 with <50 specimens are also included in the results section but these data are not used for
163 transfer functions or sea-level reconstructions.

164 A series of transfer functions based on surface samples of testate amoebae were
165 applied to the fossil data using the program C2 (Juggins, 2003). Two main data sets were
166 used for this. First, we tested several different transfer functions based on data from two
167 marshes in the region, consisting of 29 surface samples from the Little River Marsh
168 (approximately 4 km from the Wells core site) and the Little Dipper Harbour marsh in New
169 Brunswick (Gehrels et al., 2006). These data sets are illustrated in Figure 2 as taxon
170 abundance plotted against surface elevation and show the strong zonation of taxa along the
171 tidal gradient. We also applied a local transfer function to the Wells data based only on the
172 17 samples from the Little River Marsh, although this has the disadvantage that sample
173 numbers are too low to generate sample specific error estimates. Second, we tested a
174 similar series of transfer functions based on modern data from the United Kingdom
175 (Charman et al., 2002) to test whether taxon-environment relationships are robust across the
176 North Atlantic region. If modern relationships are spatially robust, it is more likely that the
177 transfer functions are also temporally robust. To assess this, the best performing transfer
178 function in cross validation of the UK data was applied to the modern North American
179 samples as well as to the fossil samples. In order to combine data from sites with different

180 tidal ranges, elevation data were normalised in relation to the difference between mean tidal
181 level (MTL) and highest astronomical tide (HAT).

182 The chronologies for the cores were based on radiocarbon, ^{210}Pb , ^{137}Cs , $^{207}\text{Pb}/^{206}\text{Pb}$ ratios
183 and pollen analyses (Gehrels et al., 2002; 2005). For Chezzetcook, the age-depth model is
184 based on ^{210}Pb ages for the upper part of the profile, cross-checked against $^{207}\text{Pb}:^{206}\text{Pb}$ ratios
185 and ^{137}Cs , and extrapolated for ages prior to c. AD 1890 to a pollen marker at AD 1780 ± 20
186 years (Gehrels et al., 2005, Figure 8b). For Wells, the chronology is based on ^{210}Pb cross
187 checked against ^{137}Cs and extrapolated to a pollen marker at AD 1760 (Gehrels et al., 2002,
188 Figure 2). The samples used for testate amoebae analysis date almost entirely from the
189 sections of the cores dated by ^{210}Pb to post AD 1900, supported by ^{137}Cs and $^{207}\text{Pb}:^{206}\text{Pb}$
190 ratios. For the Wells core, we also tested for the presence of spheroidal carbonaceous
191 particles (SCPs), markers for industrial pollution found widely in lake and peat sediments but
192 not reported from saltmarsh deposits (Rose, 2001).

193

194 **3. Results**

195 ***Effect of KOH treatment***

196 A total of 60 tests were counted in the eight samples prepared by standard water-based
197 techniques (Table 1). Both the count (29) and the concentration (56.4 mg^{-1}) were highest in
198 the sample from 8-9 cm depth, with other samples having counts between 1 and 8 tests,
199 representing concentrations of $4.9\text{-}18.1 \text{ tests mg}^{-1}$. Counts were higher overall in the samples
200 treated with KOH (71 tests counted) and concentrations were also increased in the samples
201 from 32 cm and above where counts were highest. Deeper samples had very low counts
202 (<10) and concentrations ($<10 \text{ mg}^{-1}$) in both treatments and generally showed reduced
203 counts and concentrations following KOH treatment. The assemblage composition of the
204 samples with counts >10 is very similar between treatments with most of the same dominant
205 taxa present. Furthermore, although we did not quantify counting effort, the slides were
206 generally clearer with better dispersal of material and reduced amounts of fine organic
207 material visible. We conclude that the weak 5% KOH treatment improves overall quality of

208 slides without selective effects on the assemblage. Increased concentrations in the KOH
209 treatment suggest that tests are hidden by detritus in the water-based treatment. Although
210 the numbers and range of samples and tests counted was not adequate to be conclusive, the
211 results also indicate that damage to tests was not significant enough to prevent detection and
212 identification of all taxa. Hendon and Charman (1997) also reported increased concentrations
213 of tests following a stronger 10% KOH treatment, but found significant damage to tests. Our
214 results suggest a lower concentration of KOH retains the benefit of reduced detritus while
215 avoiding problems of test damage. This is an area still requiring further work and a
216 systematic experiment to determine optimum combinations of KOH concentration,
217 temperature and length of treatment. It seems likely that weak alkali treatments of
218 problematic samples with low test concentrations improves counting efficiency and increases
219 the numbers of samples from which data can be obtained, but we would not advocate using
220 this process in the absence of some evaluation of its impact on specific samples and
221 assemblages.

222

223 **Modern assemblages and transfer functions**

224 *North American data*

225 The modern assemblages (Figure 2) show a strong zonation from elevations below mean
226 higher high water (MHHW) extending to well above highest astronomical tide (HAT) and
227 zonation is similar for both sites. A full discussion of the modern assemblages is given by
228 Gehrels et al. (2006), but we include these data here to allow comparison with the fossil
229 assemblages. The highest occurrence of foraminifera is at (Little River) or just below (Little
230 Dipper) HAT, where dominant testate amoebae are *Centropyxis cassis* type, *Euglypha*
231 *rotunda* type and *Tracheleuglypha dentata*. The lowest samples are dominated by
232 taxa such as *Digglugia pristis* type and *Centropyxis platystoma* type

233

234 Transfer functions based on weighted averaging performed best in leave-one-out cross
235 validation (Table 2), with no improvement from using a partial-least-squares version of
236 weighted averaging. A linear-based partial-least-squares model performed similarly and two
237 approaches based on modern analogue matching were significantly worse. The weighted
238 average model based on classic deshrinking (WA_Cla) had the highest r^2 (0.85), lowest
239 maximum bias and low RMSEP and was applied to the fossil data sets. For comparison with
240 a tolerance downweighted model, which is potentially more stable in reconstructions, the
241 WAT_Cla model was also applied.

242

243 *UK data*

244 The UK data set consists of modern samples from three sites (Taf estuary, S.Wales; Erme
245 estuary, Devon; Brancaster marsh, Norfolk). Previously these data were used to examine a
246 variety of relationships between testate amoebae and environmental conditions (Charman et
247 al., 2002) and to test the potential of multi-proxy microfossil data for sea-level reconstructions
248 (Gehrels et al., 2001). Here we test the ability of these data to estimate the known elevation
249 of the modern samples from the North American marshes in order assess the applicability of
250 transfer functions over very wide geographical regions..

251

252 The transfer functions for the UK data suggest very good performance of some models
253 (Table 3). In particular, the linear partial least squares (PLS) model produced very high r^2
254 (0.99) and low RMSEP (0.012) values. The modern analogue technique also produced good
255 cross validation statistics ($r^2=0.61$, RMSEP=0.063), but the residuals were systematically
256 biased with strong over-prediction in low elevation samples and under-prediction in high
257 elevation samples (plot not shown). The best performing weighted average model (WAT_Inv;
258 tolerance downweighted weighted average with inverse deshrinking) had a good r^2 (0.47)
259 and low RMSEP (0.074). Both the PLS and WAT_Inv models were applied to the US
260 samples to assess their applicability to North American data (Table 3, Figure 3).

261 Performance in terms of RMSEP (Table 3) was surprisingly good for both transfer functions,

262 although both tended to show a systematic bias towards over prediction at low elevations
263 and under-predictions at higher elevations (Figure 3a). This tendency was much more
264 extreme for the PLS model.

265
266 Normalising the data to the height between MTL and HAT is necessary in order to take
267 account of differing tidal range between sites for transfer-function development based on
268 more than one site, but for reconstruction of past sea level, these values are converted back
269 to actual elevation using the modern tidal range for the site concerned. It is assumed that
270 tidal range remained unchanged, which is reasonable for reconstructions covering only the
271 last 100 years. We therefore tested the ability of different transfer functions to estimate the
272 actual elevation for the modern samples at the US sites, as an improved test of model
273 performance (Figure 3b). The tendency of the PLS model to underestimate the difference in
274 elevation between samples was even more marked here, whereas the WAT_Inv model either
275 slightly under-estimated (Little River) or slightly over-estimated (Little Dipper) this difference.
276 Although the PLS model shows consistently higher statistical performance in cross validation
277 (Table 3) than the WAT_Inv model, the WAT_Inv model is therefore a much more robust
278 model for reconstructions. In particular, the characteristics of the WAT_Inv model mean that
279 it is much more likely to give accurate estimates of multi-decadal *trends* in reconstructions
280 from fossil samples, a key aim in high temporal resolution studies (e.g. Gehrels et al., 2002;
281 2005; 2008). Both models systematically underestimate elevation for the Little Dipper site
282 and slightly overestimate elevation for the Little River marsh, suggesting the normalisation is
283 not accurate, perhaps due to inaccuracy of the elevations for MTL and especially for HAT.
284 The UK WAT_Inv model was applied to the Wells fossil data using a correction based on the
285 average over-prediction for the Little River modern samples. No correction was applied to
286 Chezzetcook fossil samples, as no modern data were available to estimate the prediction
287 offset at this site.

288

289 **Sea-level reconstructions**

290 Wells

291 Tests were present in the top 19 cm of the Wells core, with only very low numbers below this
292 level (Figure 4, Table 1). Concentrations were up to 30,000 tests g⁻¹, but were generally
293 much lower at 5-13,000 tests g⁻¹ and only 1000-2500 tests g⁻¹ in the samples 13-16 cm
294 depth. Below 16 cm concentrations fell to 100-500 tests g⁻¹ and no tests were found in a
295 sample from 22-23 cm despite counting 200 *Lycopodium* spores. For the purposes of
296 comparison with tide-gauge data, we focused on the top part of the profile which is dated to
297 the 20th century. Details of the chronological methods are given in Gehrels et al. (2002), but
298 for this site we also tested the use of SCPs for dating. The SCP curve shows the first major
299 consistent presence of SCPs at about 12cm depth, dated to c. AD 1866 by extrapolating
300 between the pollen marker and the lowermost reliable ²¹⁰Pb ages. A mid-19th century age is
301 typical for the start of SCP curves in Europe, but this has not yet been established for North
302 America. Given the lack of sites with independently estimated ages for SCP curves, it is not
303 yet possible to use SCPs as an absolute age marker in this region. However, if adopted more
304 widely for sediment dating it would provide a valuable relative age marker and ultimately a
305 useful additional chronological tool for eastern North America.

306 In the lower part of the profile (9-16 cm depth), the testate amoeba assemblage is dominated
307 by *Centropyxis cassis* type and above 9 cm depth *Arcella catinus* type and *Diffugia pristis*
308 type are the most abundant taxa. Other more minor taxa occur throughout the profile, notably
309 other *Centropyxis* and *Diffugia* types, *Cyphoderia ampulla*, *Corythion/Trinema* types and
310 *Tracheleuglypha dentata*. All these taxa have been previously reported from this or other
311 North American marshes (Gehrels et al., 2006), with the exception of *Campascus* type,
312 previously only reported from the United Kingdom (Charman et al., 2000a).

313 With the exception of one sample, total test counts exceeded 50 for the nine
314 samples representing the 20th century, and reconstructions based on transfer functions were
315 carried out on these samples (Figure 5). All the transfer functions suggest a generally rising
316 trend in sea level through the 20th century. However, the uppermost samples suggest a
317 decline in reconstructed sea level, related to the appearance of small numbers of testate

318 amoebae typical of very high marsh or freshwater conditions (e.g. *Centropyxis arcelloides*
319 type, *Trinema lineare* type)) as well as a reduction in *Diffflugia pristis* type (Figure 4). This is
320 in opposition to the trends in the tide-gauge data which suggest a slight rise or flattening in
321 sea level during this time. The local transfer function shows lower amplitude fluctuations
322 during this period and the tolerance downweighted version of the transfer function also
323 reduces the more extreme values. The bootstrapped error estimates for the testate amoebae
324 are approximately two thirds of those for the foraminifera and the tide-gauge data fall within
325 the estimated reconstruction errors for both testate amoebae and foraminifera based
326 reconstructions, with the exception of the sample from the mid-1970s. This sample has
327 consistently lower values for sea level but there were no foraminifera data available from the
328 original counts made by Gehrels et al. (2002), so that it remains uncertain whether the
329 deviation away from tide-gauge data is a result of problems with the testate amoebae data or
330 of some factor that affected the core location itself (such as localised freshwater ingress).
331 Excluding this sample, the estimated rates of sea-level rise vary between 1.62 ± 0.17 and
332 2.20 ± 0.25 mm yr⁻¹ for the testate amoebae reconstructions, compared with 1.86 ± 0.12 mm
333 yr⁻¹ for the tide-gauge data. Although there are a number of issues raised by the testate
334 amoebae data, clear conclusions are difficult given the relatively low sediment accumulation
335 rate and low sample numbers resulting from this (9 samples from the 20th century for testate
336 amoebae and only 5 for foraminifera).

337

338 *Chezzetcook*

339 Counts exceeding 50 tests were made down to 29 cm depth, with samples covering most of
340 the 20th century (Gehrels et al., 2005). However, obtaining counts for some of these samples
341 required counting of multiple slides with considerable input of time. Concentrations are
342 similar to those at Wells with numbers up to 25,000 tests g⁻¹, but often around 5-10,000 tests
343 g⁻¹ (Figure 6). The assemblages are dominated by *Centropyxis cassis* type and *Arcella*
344 *catinus* type with lower diversity of minor taxa than at Wells. *C. platystoma* type is more

345 abundant towards the surface and *Trigonopyxis arcula* type is notable as a high marsh
346 indicator for consistent occurrence in the deeper samples.

347 The reconstructions The testate amoebae-based reconstructions are very similar to
348 each other. The tolerance downweighted model reduces the amplitude of change by only a
349 small amount and the reconstruction based on the UK data is indistinguishable from the two
350 reconstructions based on North American data (Figure 7). The overall trend in the testate
351 amoebae reconstructions is very similar to changes inferred from foraminifera although the
352 gradient in the testate amoebae record is slightly steeper (3.70 ± 0.61 to 4.07 ± 1.00 mm yr⁻¹
353 compared to 3.32 ± 0.43 mm yr⁻¹). The estimated errors for individual samples in both
354 records are very similar (± 5 cm). It is surprising that the errors are so similar, given that the
355 testate amoebae reconstructions are based on data from sites ranging from other parts of the
356 eastern North American coast and the United Kingdom. Whilst we would expect smaller
357 errors for a location with a small tidal range, it is surprising that these very different
358 approaches yield similar error estimates. Reconstructions are in reasonable agreement with
359 the tide-gauge record, which shows a linear trend of 3.3 ± 0.11 mm yr⁻¹. Larger short-term
360 fluctuations are shown by the foraminifera-based reconstruction than in the amoebae record.
361 While this may be partly due to the larger number of foraminifera samples analysed, the
362 short-term decline in sea level at c. AD 1955 registered by the foraminifera is at least partly
363 sampled but not reflected by the testate amoebae record. As this deflection is also not shown
364 by the tide-gauge record, it must be a result of changes only in foraminifera assemblages.

365

366 **4. Discussion**

367 ***Test concentrations and preparation***

368 The data on testate amoebae presented here and other previous studies highlight the
369 problems of separating low numbers of testate amoebae from a matrix of similar size
370 particles (Roe et al., 2002). In contrast to terrestrial peat, tests are often present in relatively
371 low concentrations and are obscured by large amounts of silt-sized organic and inorganic
372 detritus. We have attempted to increase the range of samples that can be economically

373 analysed by using a weak alkali treatment in preparation. This has been effective in
374 improving counts to statistically useful levels for the two cores presented here. While it is well
375 known that strong chemical pre-treatment such as that applied in pollen analysis can
376 selectively destroy tests (Tolonen, 1986; Hendon and Charman, 1997), short periods in weak
377 alkali (5% KOH in this case) do not appear to have this effect. Although further work on a
378 wider range of samples is still needed, the gains in analysis of salt-marsh sediments may
379 outweigh any concerns over loss or damage to tests. Counting of salt-marsh samples is still
380 more difficult than for many terrestrial peat samples and it takes considerable time (typically
381 0.5-1 day per sample) using multiple slide preparations to achieve counts >50 tests. Some
382 samples will remain impossible to count to these levels, and on the basis of the data from
383 Wells, this limit seems to be around concentrations of 1000 tests g⁻¹. It is not surprising that
384 fossil samples in these cores have relatively low concentrations because the cores are from
385 the high marsh within the zone dominated by *Balticammina pseudomacrescens* and
386 *Jadammina macrescens*, with low abundances of various other taxa (Gehrels et al., 2002,
387 2005). The Wells core is from 1.70m NGVD which is in the lowermost zone of testate
388 amoebae occurrence (Figure 2). Testate amoebae are most abundant and diverse above
389 this zone (Charman, 1998; Gehrels et al., 2001) so the core location may be sub-optimal for
390 testate amoebae populations. The Chezzetcook core is from just below HAT, so is in a better
391 position; this may be one of the reasons why the reconstruction for this site shows greater
392 agreement with the tide gauge record.

393

394 ***Regional scale transfer functions***

395 The application of the UK training set to modern and fossil North American samples shows
396 that testate amoebae zonation is sufficiently consistent over large regions such as the North
397 Atlantic, to be able to use regional scale transfer functions successfully. Elevations estimated
398 for North American modern samples using the UK transfer function are surprisingly accurate
399 (Table 3, Figure 3). Reconstructions from fossil samples show very little difference with those
400 based on the North American transfer function (Figures 5 and 7). This is in contrast to

401 foraminifera, where generally application is limited to local (Gehrels et al., 2001) or regional
402 (Horton et al., 1999b; Edwards et al., 2004) training sets. The wide geographical applicability
403 of the transfer functions suggests that they are more likely to be temporally robust than
404 transfer functions that only perform well in cross validation for single locations or small
405 regions. Whilst our data are still relatively few, these results indicate excellent potential for
406 developing large scale regional training sets in future work. Testing transfer functions by
407 application to other regions also reveals some of the problems with relying on cross-
408 validation statistics to evaluate model performance (Figure 3). Whilst extremely good
409 performance can sometimes be shown for some models such as the PLS model for the UK
410 data (Table 3), problems with systematic bias are clearly revealed when using them to
411 estimate elevations of independent samples with known elevations (Figure 3). It seems likely
412 that transfer functions that make use of residual variation in each iteration of the calibration
413 calculation (PLS, WAPLS) will be more susceptible to such problems.

414

415 ***Precision and accuracy of reconstructions***

416 One of the main aims in pursuing work on testate amoebae in salt marshes is to improve
417 precision of palaeo-sea level estimates. However, more precise reconstructions must also be
418 demonstrably accurate. Estimated sample-specific errors in fossil samples derived from
419 bootstrapping of the models based on the modern North American data are around ± 5 cm for
420 Chezzetcook and ± 11 cm for Wells, the difference being due to the larger tidal range at
421 Wells. However, these error terms only include uncertainty in the relationship between
422 modern assemblages and measured elevation. They assume the relationship between fossil
423 assemblages and palaeo-elevation is the same, disregarding possible effects of changing
424 tidal regimes, disturbance, taphonomy, preservation and processing, and any other factors
425 that may have influenced the observed assemblage. Good agreement between proxies and
426 tide-gauge data in the Chezzetcook sequence suggests that these other effects are small at
427 least for the last 100 years, and thus we have greater confidence in the envelope of
428 uncertainty at this site (Figure 7). The agreement is less strong for the Wells site, although

429 low sedimentation rates and consequently low sample resolution make clear conclusions
430 difficult here (Figure 5). The similarity of error estimates for the UK and North American
431 transfer functions also supports the idea that local site factors play only a relatively minor role
432 in determining testate amoebae assemblages.

433

434 An alternative approach to assessing precision is to calculate RMSEP values based on a
435 comparison between the reconstructed values and the tide gauge data. This yields more
436 realistic error estimates than those based purely on cross validation. In this case, the
437 RMSEP values are 6.7 cm for the Wells WAT reconstruction and 3.5 cm for the Chezzetcook
438 reconstruction. Surprisingly the RMSEP values for both cores are lower than the
439 bootstrapped RMSEP values suggesting the actual error is less than that estimated from the
440 transfer function alone, and supporting the idea that bootstrapped errors can be used as a
441 reasonable conservative estimate of the average actual error in reconstructions from the pre-
442 instrumental period. However, it is also clear that some individual samples can yield
443 reconstructed values that are clearly out of line with recorded variations and, if taken at face
444 value in the fossil record, would imply rapid short-term fluctuations in past sea level. Clearly
445 estimates of past sea-level change are more robust for multi-decadal to centennial trends
446 than they are for decadal fluctuations, even where sedimentation rates allow for such high
447 resolution reconstructions.

448

449 **5. Conclusion: Improving sea-level reconstructions from testate amoebae**

450 The cores used here for analysis were initially selected to optimise results from foraminiferal
451 analysis. They are within the most stable area of the high salt marsh within the narrow zone
452 dominated by *Balticammina pseudomacrescens* and other high marsh foraminifera. In this
453 zone foraminifera are abundant at the surface and down-core. However, testate amoebae
454 are most abundant and most clearly zoned in the very uppermost parts of salt marshes
455 (Charman et al., 2001; Gehrels et al., 2006). The fossil assemblages recovered in the two
456 cores presented here are indicative of the mid to lower zones of testate amoebae occurrence

457 in marshes. There are very few of the taxa that are associated with higher locations on the
458 marsh, where zonation of assemblages is better defined because of rapid turnover of taxa
459 along the elevation gradient (Charman et al., 1998; 2002). Coring in locations at higher
460 elevations than would be used for foraminiferal analyses may yield more diverse faunas and
461 perhaps better constrained estimates of past sea levels. The setting of the highest part of the
462 marsh means that these zones are some of the best locations for sea-level reconstruction
463 because they are geomorphologically less dynamic than lower zones. Sedimentation rates
464 are likely to be stable, with continuous sediment accumulation over time. Thus, it is
465 particularly worthwhile pursuing proxy sea-level indicators such as testate amoebae in these
466 locations.

467

468 Despite the difficulties with small surface-training sets and sub-optimal core locations, the
469 Chezzetcook record shows that testate amoebae can be used to reconstruct past sea levels
470 with high precision and accuracy. The agreement with both foraminiferal reconstructions and
471 tide-gauge data shows that this technique is promising. The precision of the reconstructed
472 values is approximately ± 5 cm in this microtidal location as assessed by cross validation of
473 modern training sets and somewhat lower (RMSEP 3.5 cm) when compared with tide gauge
474 data. This is similar or slightly better than the precision obtained from foraminifera, but this
475 level of precision is unusual for such studies. Furthermore, the testate amoebae data were
476 from a range of locations on the eastern North American coast and the United Kingdom,
477 suggesting testate amoebae are highly robust against local variations in the species-
478 environment relationships used to estimate past sea levels.

479

480 The nominal precision as estimated by cross validation in the Wells reconstruction is better
481 than that for foraminifera. This is likely due to the macrotidal location where foraminifera
482 zones are less tightly constrained. However, RMSEP values calculated from comparison with
483 the tide gauge records are slightly higher for testate amoebae than for foraminifera. If the
484 niches of testate amoebae taxa can be better constrained by further studies on surface

485 samples so that the accuracy of estimates is improved, higher precision estimates of past
486 sea-level change based on testate amoebae analysis will be possible at further sites. Future
487 studies should retain multi-proxy indicators as a cross check on the accuracy of
488 reconstructions, as well as utilising tide-gauge records alongside high resolution
489 reconstructions to test reconstruction methodologies. Careful evaluation of transfer functions
490 using approaches other than cross-validation of modern data sets is essential to avoid over-
491 estimating the ability of these techniques to reconstruct past changes from fossil samples.
492 Comparison with tide gauge records provides a suitable means of achieving this.

493

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499

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Depth (cm)	Count	T. lineare	C. dubium	D. pristis	D. pulex	D. type	P. fulva	C. Cassis	Cyphoderia	A. catinus	E.rotunda	Unknown	Total
8-9	29	1.9		7.8	3.9	11.7			1.9	27.2		1.9	56.4
16-17	6							1.2		4.6		1.2	7.0
24-25	4			2.8			5.6	2.8					11.2
31-32	3		6.0					6.0				6.0	18.1
40-41	1											4.9	4.9
48-49	3									4.9			4.9
57-58	6					2.6	2.6			2.6			7.9
65-66	8					1.9				5.7			7.6
Total	60	1.9	6.0	10.6	3.9	16.2	8.2	10.0	1.9	45.1	0.0	14.0	117.9

5% KOH

Depth (cm)	Count	T. lineare	C. dubium	D. pristis	D. pulex	D. type	P. fulva	C. Cassis	Cyphoderia	A. catinus	E.rotunda	Unknown	Total
8-9	41			23.3	5.8				5.8	44.7			79.7
16-17	13			4.6					3.5	7.0			15.1
24-25	12			8.4		2.8		8.4			8.4	5.6	33.5
31-32	4	6.0				12.1						6.0	24.2
40-41	0												
48-49	0												
57-58	1						1.3						1.3
65-66	0												
Total	71	6.0	0.0	36.3	5.8	14.9	1.3	8.4	9.3	51.7	8.4	11.6	153.7

Table 1: Testate amoebae counts from standard (upper) and KOH-based (lower) sample pre-treatment for eight samples from the Wells core. Total no.tests counted in left hand column. All other figures are concentrations (nos. mg⁻¹ wet weight sample).

Model	r^2	Average bias	Max. bias	RMSEP
WA_Inv	0.84	-0.0027	0.104	0.053
WA_Cla	0.85	-0.0034	0.084	0.054
WAT_Inv	0.80	-0.0047	0.123	0.059
WAT_Cla	0.81	-0.0055	0.099	0.060
PLS	0.81	-0.0011	0.095	0.057
MAT	0.78	-0.0173	0.166	0.068
WMAT	0.80	-0.0120	0.124	0.061

Table 2: Transfer function performance in cross validation using the leave one out (jack-knifing) method for the North American testate amoebae data. Results of WAPLS were the same as for simple weighted averaging and are not shown here. RMSEP (Root mean squared error of prediction) and bias data are for elevation normalised to the difference between mean tide level and highest astronomical tide.

Model and application	r²	RMSEP
<i>a) Cross validation</i>		
WA_Inv (Weighted average, inverse deshrinking)	0.41	0.076
WA_Cla (Weighted average, classical deshrinking)	0.43	0.098
WAT_Inv (Tolerance downweighted weighted average, inverse deshrinking)	0.45	0.074
WAT_Cla (Tolerance downweighted weighted average, classical deshrinking)	0.47	0.104
WAPLS Component 3 (Weighted average partial least squares)	0.42	0.077
PLS Component 5 (Partial least squares)	0.99	0.012
MAT (Modern analogue)	0.44	0.074
WMAT (Weighted modern analogue)	0.61	0.063
<i>b) Applied to US data</i>		
WAT_Inv		0.083
PLS Component 5		0.101

Table 3: Model performance results for the UK testate amoebae data; a) in cross validation using normalised (MTL-HAT) data, b) applied to normalised modern US data. , c) applied to actual elevation above MTL for individual sites. For b) and c), the RMSEP is calculated on the errors between the observed and predicted values shown in Figure 3, not on the linear trend lines shown.

Figure captions

Figure 1: Locations for the core sites. See Gehrels et al. (2006) and Charman et al. (2002) for details of modern sampling sites.

Figure 2: Modern testate amoebae data plotted against elevation from the marshes used in the North American transfer function. a) Little River marsh, Wells, Maine, b) Little Dipper Harbour Marsh, New Brunswick. The tidal data shown on the height axis are highest astronomical tide (HAT) and mean highest high water (MHHW). HF is the maximum height of foraminifera recorded in the 63-500 μm size range. .

Figure 3: Comparison between observed sample elevations for the modern North American samples and their predicted elevations based on the UK transfer function applied to the testate amoebae assemblages. a) For all samples plotted as elevation normalised for tidal range (MTL-HAT). b) and c) For untransformed elevations in height above MTL, b) Little River and c) Little Dipper Harbour. Circles – Little River, Squares – Little Dipper, Open – PLS model, Closed – WAT model. Linear regression lines shown as black (Little River) and grey (Little Dipper). Grey dashed line – 1:1 line.

Figure 4: Testate amoebae diagram for core FS-1, Wells, showing all samples, including those with low total counts. A further sample at 22.5 cm contained no testate amoebae or foraminifera. The estimated ages are shown with 2σ errors based on ^{210}Pb analyses.

Figure 5: Comparison between reconstructions for the Wells core based on testate amoebae, foraminifera and the tide gauge record from Portland, Maine (grey symbols). The rates of sea-level change are the linear trends over the period covered by the tide-gauge record. The rates for testate amoebae exclude the sample from the mid-1970s which gives consistently

lower values and is not represented in the foraminifera samples. For comparison, the linear trend of the tide-gauge record is $1.86 \pm 0.12 \text{ mm yr}^{-1}$. Vertical error bars are RMSEP from transfer functions. Age error bars are 2σ errors from age-depth model. No RMSEP values calculated for c) because of the low number of samples ($n=17$) in the local training set.

Figure 6: Testate amoebae % diagram from Chezzetcook core CZ-25.

Figure 7: Sea level reconstructions based on testate amoebae and foraminifera from Chezzetcook Marsh compared to the tide gauge record from Halifax (grey symbols). The rates of sea-level change are the linear trends over the period covered by the tide-gauge record. For comparison, the linear trend of the tide-gauge record is $3.30 \pm 0.11 \text{ mm yr}^{-1}$. Vertical error bars are RMSEP from transfer functions. Age error bars are 2σ errors from age-depth model.

Figure

[Click here to download Figure: Figure 1.pdf](#)

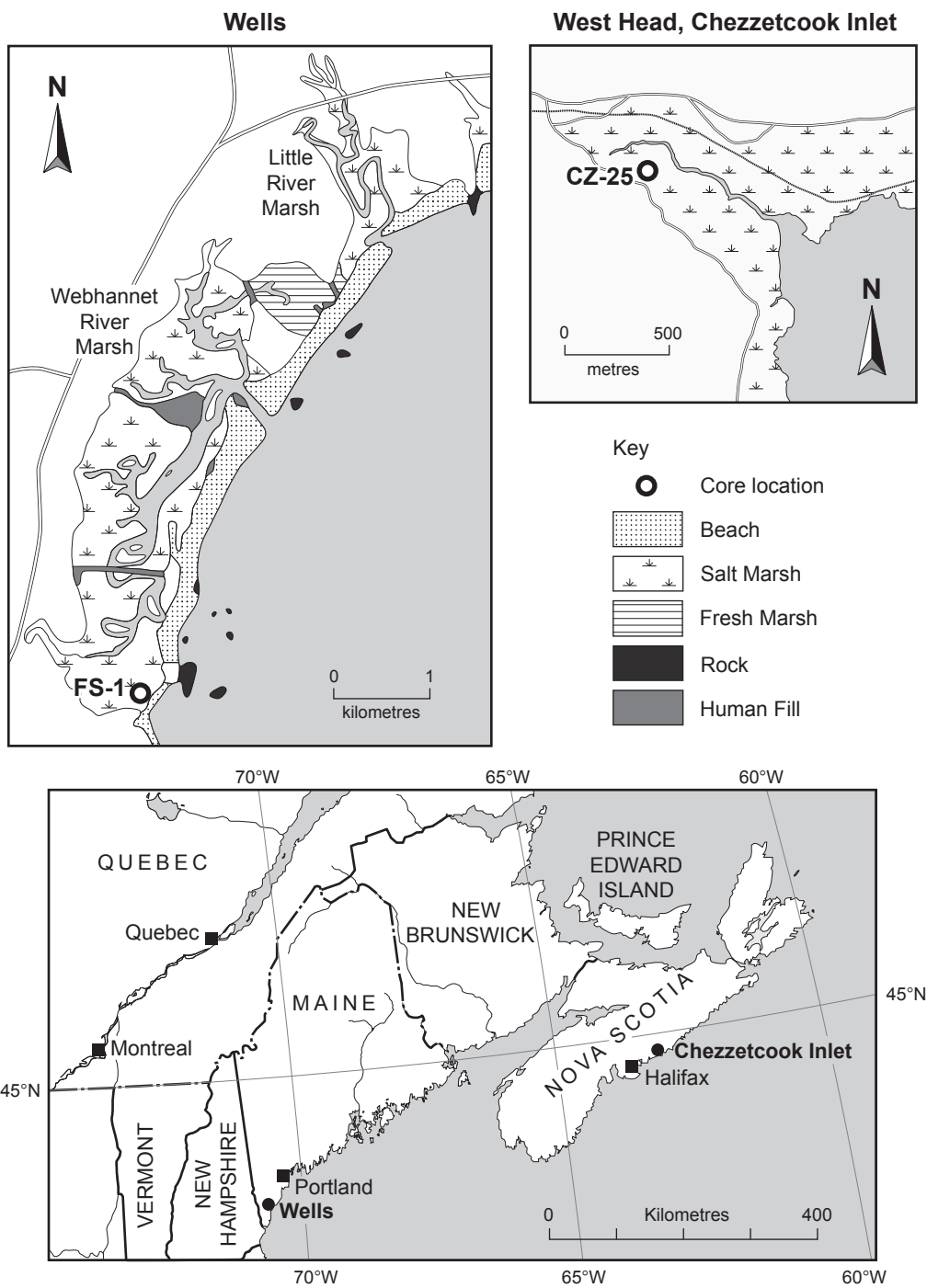


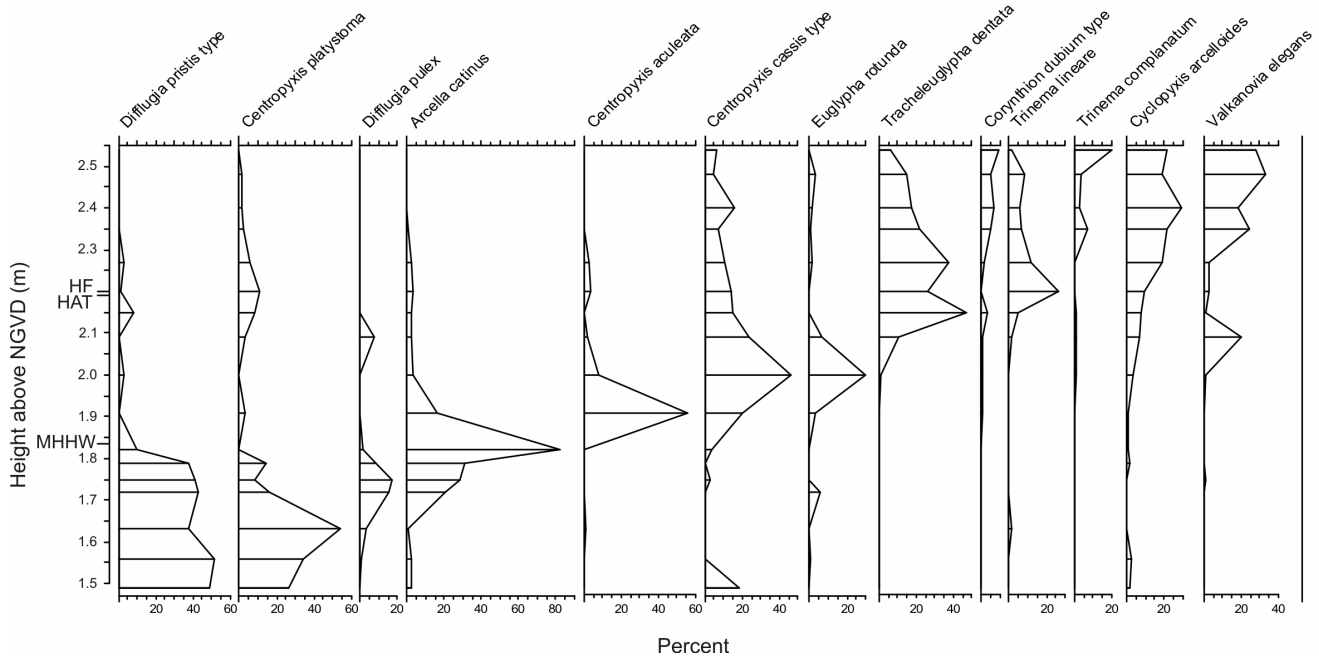
Figure 2

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a)

Little River marsh, Wells, Maine

Testate amoebae



b)

Little Dipper Harbour marsh, New Brunswick

Testate amoebae

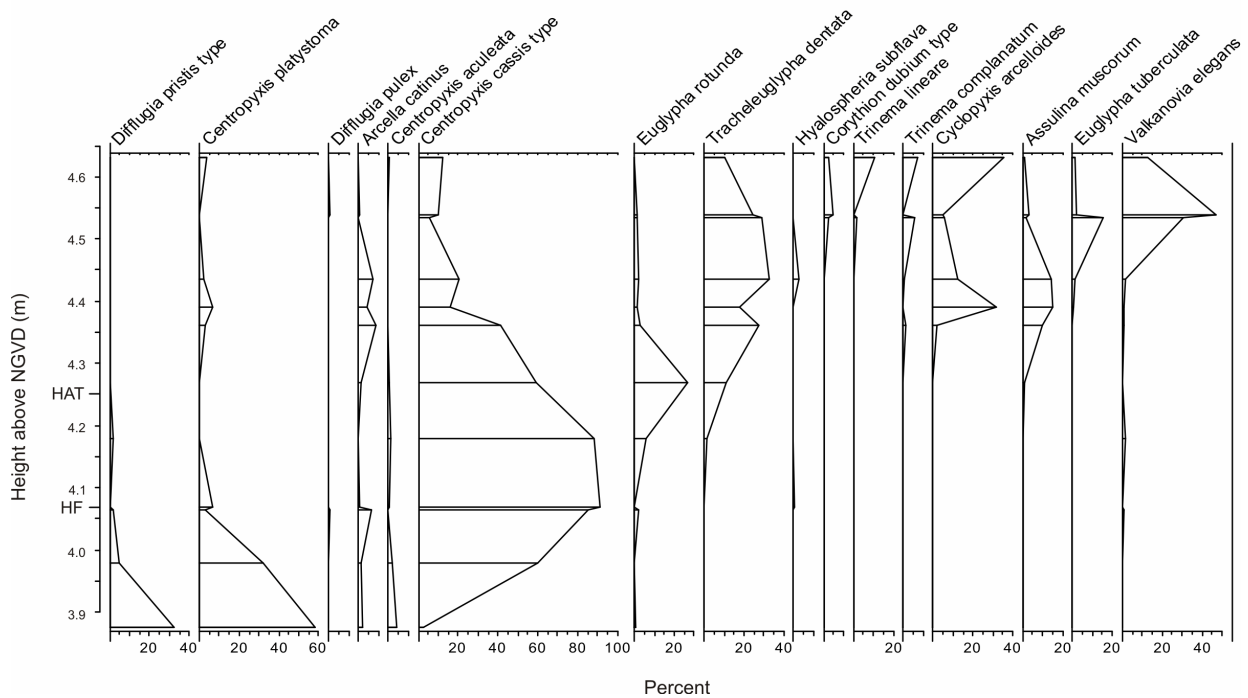


Figure 3
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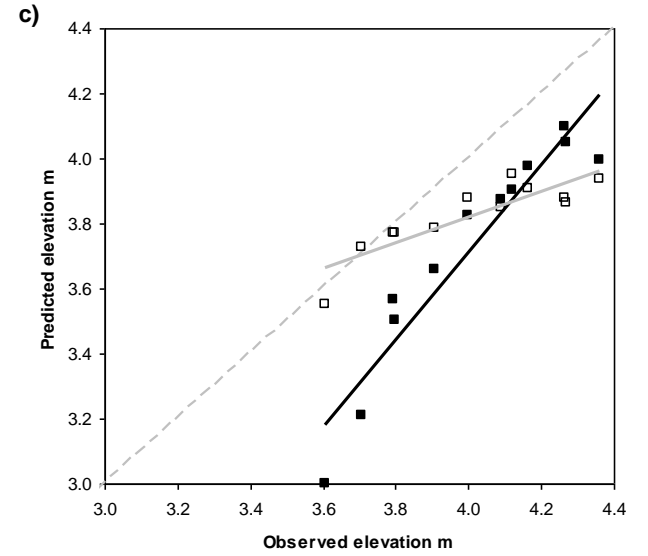
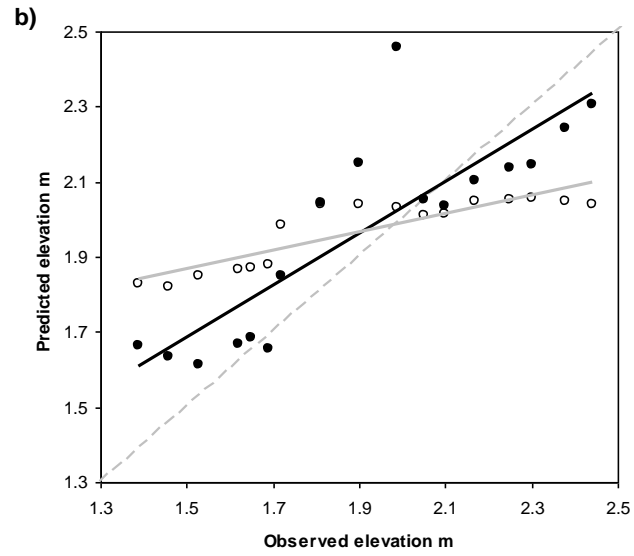
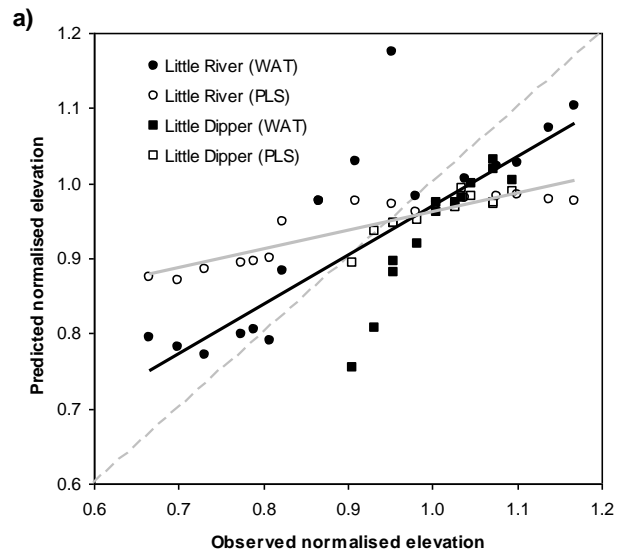


Figure 4
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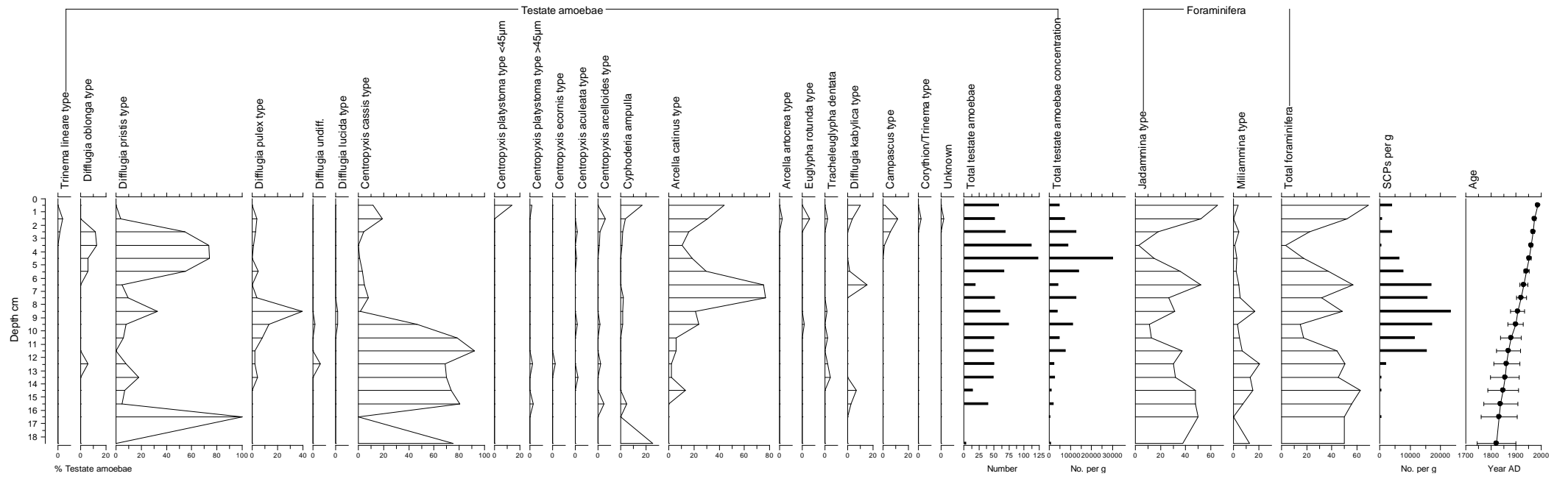


Figure 5

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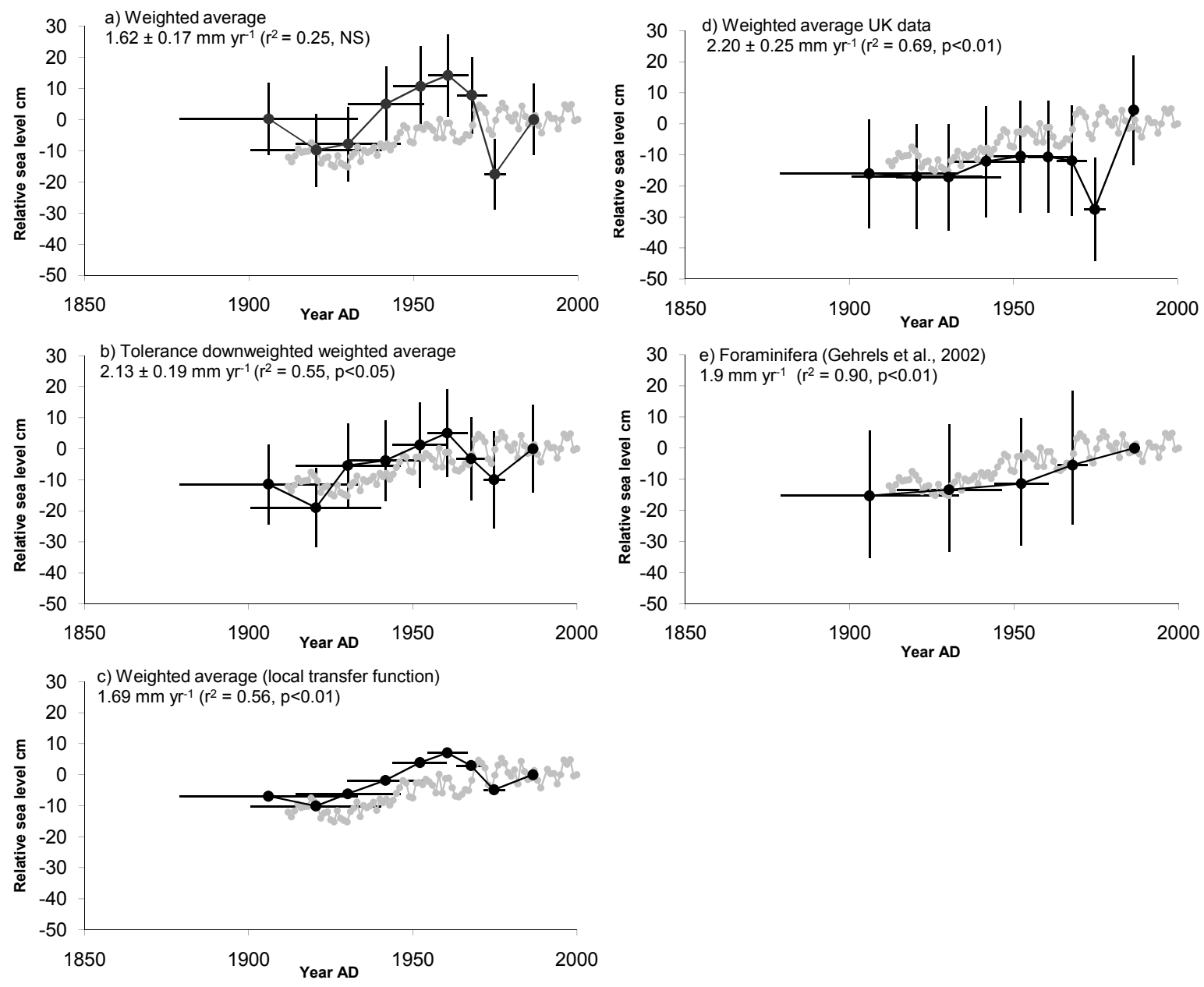


Figure 6
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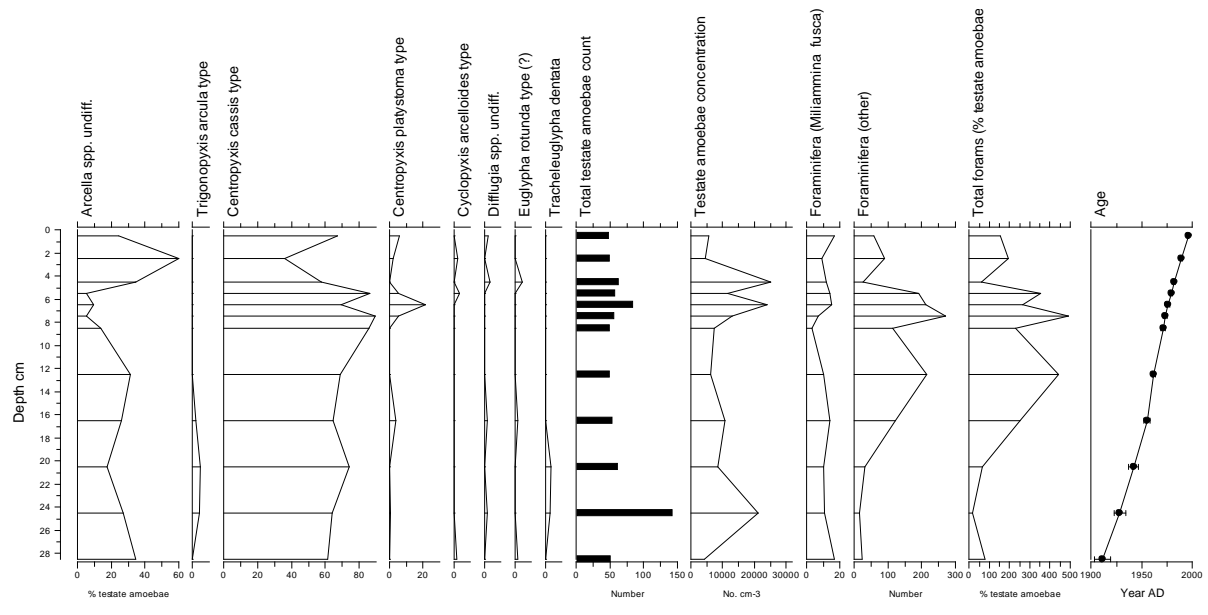


Figure 7

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