UTILITY OF SALT-MARSH FORAMINIFERA, TESTATE AMOEBAE AND BULK-SEDIMENT δ^{13}C VALUES AS SEA-LEVEL INDICATORS IN NEWFOUNDLAND, CANADA

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24   **ABSTRACT**

25

26   We investigated the utility of foraminifera, testate amoebae and bulk-sediment $\delta^{13}C$ measurements for

27   reconstructing Holocene relative sea level from sequences of salt-marsh sediment in Newfoundland,

28   Canada. Modern, surface sediment was collected along transects from low to supra-tidal elevations in

29   eastern (at Placentia) and western (at Hynes Brook and Big River) Newfoundland. Consistent with

30   previous work, low-diversity assemblages of foraminifera display an almost binary division into a higher

31   salt-marsh assemblage dominated by *Jadammina macrescens* and *Balticammina pseudomacrescens* and a

32   lower salt-marsh assemblage comprised of *Miliammina fusca*. This pattern and composition resembles

33   those identified at other high latitude sites with cool climates and confirms that foraminifera are sea-level

34   indicators. The lowest occurrence of testate amoebae was at approximately mean higher high water. The

35   composition of high salt-marsh testate amoebae assemblages (*Centropyxis cassis* type, *Trinema* spp.,

36   *Tracheleuglypha dentata* type, and *Euglypha* spp.) in Newfoundland was similar to elsewhere in the

37   North Atlantic, but preservation bias favors removal of species with idiosomic tests over those with

38   xenosomic tests. The mixed high salt-marsh plant community in Newfoundland results in bulk

39   surface-sediment $\delta^{13}C$ values that are typical of C_3 plants, making them indistinguishable from freshwater

40   sediment. Therefore we propose that the utility of this proxy for reconstructing RSL in eastern North

41   America is restricted to the coastline between Chesapeake Bay and southern Nova Scotia. Using a simple,

42   multi-proxy approach to establish that samples in three radiocarbon-dated sediment cores formed between

43   the lowest occurrence of testate amoebae and the highest occurrence of foraminifera, we generated three

44   example late Holocene sea-level index points at Hynes Brook.

1. INTRODUCTION

In eastern North America, quiet-water coastal environments are occupied by salt marshes that have a robust and predictable relationship to tidal datums (e.g., Redfield, 1972). These ecosystems respond to relative sea-level (RSL) rise by accumulating sediment to ensure that the active salt-marsh surface maintains its tidal elevation, while increasing in absolute elevation relative to a fixed point of reference on land. Consequently, buried units or sequences of salt-marsh sediment preserve a history of Holocene RSL changes (e.g., Bloom and Stuiver, 1963, Stuiver and Daddario, 1963). Interrogating this sedimentary archive to reconstruct RSL relies on the use of sea-level indicators to accurately establish the elevation of dated paleomarsh surfaces with respect to contemporary and modern tidal datums (e.g., Woodroffe and Barlow, 2015).

Sea-level indicators are biological assemblages, chemical signatures or physical features with a known relationship to tides. In salt-marshes, the most commonly used sea-level indicators are plants, assemblages of microfossils such as foraminifera (e.g., Edwards and Wright, 2015, Scott and Medioli, 1978), diatoms (e.g., Zong and Horton, 1999, Zong and Sawai, 2015), or testate amoebae (e.g., Barnett et al., 2016, Charman, 2015) and bulk-sediment geochemistry (e.g., Kemp et al., 2012b, Lamb et al., 2006). The analogy between modern sea-level indicators and their paleo counterparts is the basis for estimating the paleo tidal elevation at which a fossil sample formed and subsequently for reconstructing RSL. This approach is reliant upon the availability of an empirical modern training set comprised of paired observations that establish and quantify the relationship between a sea-level indicator and tidal datums. Due to the influence of secondary environmental variables such as the prevailing climate, oceanographic and salinity regimes, it is usually necessary to develop a modern training set in the region that will be targeted for reconstructing RSL.

Since deglaciation of the Laurentide Ice Sheet (by ~7000 years before present; BP, 1950 CE), the principal cause of RSL change along the Atlantic coast of North America was ongoing and spatially-variable glacio-isostatic adjustment (GIA). Earth-ice models predict that the hinge line between regions experiencing recent RSL rise/fall from GIA occurs close to the Gulf of St. Lawrence (Figure 1; e.g., Peltier, 2004). Newfoundland is therefore one of the most northerly regions to preserve a salt-marsh record of RSL rise since the mid-Holocene, which makes it an important location for investigating drivers of paleo-RSL change that vary with latitude such as ocean dynamics (Ezer et al., 2013, Levermann et al., 2005, Yin and Goddard, 2013) and the fingerprint of Greenland Ice-Sheet melt (Mitrovica et al., 2011). We build on previous investigations of foraminifera (Daly, 2002, Daly et al., 2007) to explore the utility of foraminifera, testate amoebae and bulk-sediment $\delta^{13}C$ values as independent sea-level indicators in

79 Newfoundland and to evaluate their use as part of a multi-proxy approach. This work will support future
80 efforts to reconstruct RSL using salt-marsh sediment. At three sites (Placentia on the Avalon Peninsula
81 and Hynes Brook and Big River on the Port-au-Port Peninsula; Figure 1) we collected surface sediment at
82 regular vertical increments to establish the relationship between each type of potential sea-level indicator
83 and local tidal datums. We demonstrate that foraminifera and testate amoebae are viable sea-level
84 indicators (with some limitations), while bulk-sediment δ^{13}C values have little utility in this region. An
85 example RSL reconstruction is produced by application of these sea-level indicators to three
86 radiocarbon-dated basal salt-marsh peats from Hynes Brook spanning the last ~2000 years. These
87 reconstructions demonstrate the utility of using a simple, multi-proxy approach to identify material that
88 formed between the lowest occurrence of testate amoebae and the highest occurrence of foraminifera.
89
90   **2. REGIONAL SETTING**
91 We studied three sites in Newfoundland (Placentia, Hynes Brook and Big River; Figure 1) that were
92 identified from existing literature as having well-developed modern salt marshes and being underlain by
93 sediment (high salt-marsh peat) that was likely to yield a viable RSL reconstruction (e.g., Bell et al.,
94 2005, Brookes et al., 1985, Daly, 2002, Daly et al., 2007, Wright et al., 2011, Wright and van de Plassche,
95 2001). In some cases, these earlier studies also included analysis of surface foraminiferal assemblages.
96 These sites experience a maritime climate characterized by short, cool summers (maximum average
97 monthly warm temperature of ~20 °C in July) and winters in which the average high temperature for
98 January is approximately –2 °C (Figure 1). The prevailing oceanographic regime brings cold water (the
99 Labrador Current) to the sites resulting in an average annual sea-surface temperature of ~1 °C in the open
100 ocean.
101
102 Placentia is located on the Avalon Peninsula in eastern Newfoundland (Figure 1), where salt marshes are
103 rare. The study site lies on the protected side of a progradational barrier system comprised of vegetated
104 ridges and swales. It is indirectly connected to the ocean because the 'south arm' is closed off by a gravel
105 barrier and marine water reaches the site having first entered the back-barrier lagoon and fluvial system
106 through the open 'north arm'. This geomorphology results in a great diurnal tidal range (mean lower low
107 water, MLLW to mean higher high water, MHHW) of 0.91 m. The low gravel barrier directly in front of
108 the salt marsh is overwashed by high tides. Low salt-marsh environments are absent at the site, although
109 isolated stands of *Spartina alterniflora* are present and rooted in gravel rather than fine-grained sediment.
110 The high salt-marsh is a diverse, peat-forming community comprised of *Distichlis spicata*, *Spartina*
111 *patens*, *Juncus geradi*, *Potentilla* sp., *Plantago maritima, Glaux maritima*, and *Carex glareosa*. Within
112 the high salt-marsh zone these plants form a mosaic pattern and are frequently intermixed, although some

113 mono-specific stands are present. At the upland edge of the salt marsh is a near-vertical slope of

114 weathered bedrock that is sparsely vegetated by fir trees, pine trees and brambles. At this site, Daly

115 (2002) described foraminifera in six surface sediment samples spanning an elevational range of ~0.25 m.

116

117 On the Port-au-Port Peninsula salt marshes occupy three small incised valleys on the sheltered coast of

118 West Bay that are protected by a beach barrier/spit formation at their entrance (Figure 1). We recovered

119 samples from Hynes Brook and Big River, which both display a characteristic division of floral

120 communities into a low salt-marsh zone dominated by *Spartina alterniflora* (tall form) and a mixed high

121 salt-marsh mosaic comprised of variable combinations of *Iris versicolor*, *Schoeneplectus* spp., *Spartina*

122 *patens*, *Distichlis spicata*, *Plantago maritima*, *Triglochin maritima*, *Glyceria borealis*, *Glaux maritima*,

123 *Agrostis stolonifera*, and *Spartina alterniflora* (short form). These species are frequently intermixed,

124 although some mono-specific stands are present, for example *Iris versicolor* is often the only plant present

125 at the upper limits of tidal inundation (e.g., Daly, 2002). The great diurnal tidal range at Hynes Brook and

126 Big River is 1.06 m. At Hynes Brook, Daly (2002) described foraminifera in twelve surface sediment

127 samples spanning an elevational range of ~0.70 m. Previous studies demonstrated that Hynes Brook is

128 underlain by sequences of high salt-marsh peat spanning the last ~3000 years (Bell et al., 2005, Brookes

129 et al., 1985, Daly, 2002, Daly et al., 2007).

130

131   **3. MATERIALS AND METHODS**

132   *3.1 Modern sampling regime*

133 We described the modern distribution of foraminifera, testate amoebae and bulk-sediment $\delta^{13}C$ values

134 from surface (0–1 cm) sediment samples collected along transects at each of our three study sites (Table

135 1). Sampling stations were positioned at regular (~5 cm) vertical intervals spanning the full height range

136 occupied by salt-marsh vegetation to ensure even sampling of the environmental gradient of interest

137 (elevation). The uppermost samples of each transect were recovered from supra-tidal environments to

138 capture the highest occurrence of foraminifera (Wright et al., 2011) and the turnover of testate amoebae

139 assemblages across the upper limit of marine influence (e.g., Barnett et al., 2016).

140

141 Sample heights at each site were surveyed with a total station and are expressed relative to mean tide level

142 (MTL). Local tidal datums were determined by comparing data from on-site water-loggers (corrected for

143 the inverse barometer effect) with the closest tide gauge station operated by Fisheries and Oceans Canada.

144 At Placentia, two water-loggers were installed to quantify the distortion of the tidal wave as it propagates

145 into the back-barrier system. One logger measured water depths immediately adjacent to the salt-marsh

146 site, while a second logger was deployed in the open bay beyond the gravel barrier (Figure 1c, d). High

147 and low tides captured by the water-logger in the bay match those measured by the neighboring tide

148 gauge at Argentia in timing and amplitude. We used hourly water-level measurements from Argentia

149 spanning the current tidal epoch (1983–2001) to define tidal datums following the definitions provided by

150 the National Ocean and Atmospheric Administration (NOAA). We then used the difference in water-level

151 measurements between the salt-marsh and open-bay water loggers to establish local tidal datums at

152 Placentia by scaling those previously defined for Argentia. For Hynes Brook and Big River, we used the

153 tidal datums reported in Wright et al. (2011), who employed a similar approach to the one outlined above

154 using the long-term tide-gauge measurements from Port aux Basques (Figure 1).

155

156   *3.2 Processing and analysis of surface sediment samples*

157 Samples collected for foraminiferal analysis were placed into vials with buffered ethanol and stained with

158 rose Bengal to allow identification of individuals that were living at the time of collection (e.g., Figueira

159 et al., 2012). In the laboratory, each sample was washed over 500 µm and 63 µm sieves to isolate the

160 foraminifera-bearing fraction, from which a minimum of 100 dead individuals were counted in water

161 under a binocular microscope. We determined the highest occurrence of foraminifera to occur where the

162 concentration of dead tests decreased abruptly and above which there were insufficient individuals to

163 reasonably conclude that they represented a viable and *in situ* population. Species were identified through

164 comparison to published literature (e.g., Edwards and Wright, 2015, Scott and Medioli, 1980, Scott et al.,

165 1981, Smith et al., 1984, Wright et al., 2011) and type slides from nearby regions. Different taxonomic

166 approaches to the classification of salt-marsh foraminifera exist, reflecting the extent to which

167 morphological variation is viewed as phylogenetic or ecophenotypic. While early work combined a range

168 of forms within the taxon *Trochammina macrescens*, we differentiate between *Jadammina macrescens*

169 and *Balticammina pseudomacrescens* following (Daly, 2002), de Rijk (1995), Gehrels and van de

170 Plassche (1999), Wright et al. (2011), in recognition of their spatially distinctive distributions (see

171 Edwards and Wright, 2015 for discussion). We also distinguished between *Trochammina inflata* and

172 *Siphotrochammina lobata*.

173

174 At each sampling station ~2 cm^3 of surface sediment was collected and sealed in a bag for subsequent

175 analysis of testate amoebae following the protocol developed for salt-marsh environments (Barnett et al.,

176 2013). For each sample, 1 cm^3 of surface sediment was combined in 100 ml of deionized water with one

177 tablet of *Lycopodium clavatum* to provide an exotic marker of known concentration during counting

178 (Stockmarr, 1971). The material was heated at 80 ˚C for one hour on a hot plate and regularly stirred to

179 aid disaggregation. After being left to soak for >12 hours, the material was wet sieved and the 300-63 µm

180 fraction retained for counting. Samples were mounted onto glass microscope slides using deionized water

181   and observed under 400x magnification. We counted 100 individual tests per sample. Where test

182   concentrations were <1500 tests per cm^3, counting ceased after 100 *Lycopodium* spores were counted.

183   This point marked the lowest extent of testate amebae occurrence where counts per *Lycopodium* spore

184   decreased abruptly and below which there were insufficient individuals to reasonably conclude that they

185   represented a viable and *in situ* population. Our taxonomy is based on the systematic identification keys

186   of Charman et al. (2000) and Booth and Sullivan (2007). These keys were supplemented by published

187   literature pertaining to salt-marsh and littoral taxa (Cash et al., 1905, 1909, 1915, Charman et al., 2002,

188   Gehrels et al., 2006a, Golemansky, 1974, Golemansky and Todorov, 2004, 2007, Nicholls, 2007, 2009,

189   Ooms et al., 2015, Todorov et al., 2009).

190

191   Representative sub samples of the surface material were analyzed in duplicate for δ^{13}C, δ^{15}N, total organic

192   carbon (%), and total nitrogen (%). Sediment samples were treated with 10% HCl to remove carbonate,

193   rinsed to neutral pH with deionized water, and then dried at 50 ˚C and ground to a fine, homogenized

194   powder. The samples were analyzed using a Costech 4010 Elemental Analyzer interfaced with a Thermo

195   Delta V Plus stable ratio mass spectrometer (EA-IRMS) at the University of North Carolina Wilmington.

196   Isotopic composition is reported in standard δ -per mil notation (‰) relative to the Vienna-Pee Dee

197   Belemnite (V-PDB) standard. Presented results are the average of the duplicate measurements. Repeated

198   analyses of USGS 40 and 41 glutamic acid standards indicate that the precision of these analyses is better

199   than ± 0.5 ‰. δ^{15}N, total organic carbon (%), and total nitrogen (%)

200

201   *3.3 Relative sea-level reconstruction*

202   To investigate the utility of foraminifera, testate amoebae and bulk-sediment δ^{13}C values for

203   reconstructing RSL in Newfoundland, we collected three cores of basal salt-marsh sediment along

204   transect HBM-2 at Hynes Brook (Figure 1g). The sediments were recovered using a Russian-type corer to

205   prevent compaction or contamination, and were stored in rigid plastic sleeves, wrapped in plastic and

206   refrigerated until processing. Each core comprised a single, 50-cm long sequence that captured the

207   transition from basal consolidated gray silty clay into an overlying dark-brown to black organic silt. One

208   half of each core was sliced into 1-cm thick contiguous samples for analysis of foraminifera, testate

209   amoebae and bulk-sediment δ^{13}C. Processing of these samples followed the methods outlined for surface

210   samples with the exception of adding rose Bengal to the foraminiferal samples.

211

212   The other half of each core provided material for radiocarbon dating which was extracted from the

213   sediment matrix and cleaned following Kemp et al. (2013c). We selected only samples that were

214   deposited on, or close to, a paleo marsh surface such as small, horizontal twigs and the shallow rhizomes

215 of identifiable salt-marsh plants. The samples were analyzed at the National Ocean Sciences Accelerator

216 Mass Spectrometry facility, where they underwent standard acid-base-acid pretreatment and $\delta^{13}C$ was

217 measured directly on an aliquot of CO_2 collected during sample combustion. Reported radiocarbon ages

218 were individually calibrated using the Intcal13 dataset (Reimer et al., 2013) and we used the upper and

219 lower 2σ calibrated ages as the range of possible ages for the dated sample.

220

221 The lowest depth with a viable assemblage of foraminifera and material suitable for radiocarbon dating

222 was used to produce a sea-level index point from each core. We counted foraminifera and testate amoebae

223 and measured bulk-sediment $\delta^{13}C$ values in additional core samples surrounding the dated level to ensure

224 that it was representative of the prevailing environmental conditions at the time of sediment deposition.

225 Relative sea level was calculated by subtracting reference water level (estimated from foraminifera,

226 testate amoebae and bulk-sediment $\delta^{13}C$ values) from measured sample elevation (e.g., Woodroffe and

227 Barlow, 2015). Vertical uncertainty for the reconstruction was calculated following Engelhart and Horton

228 (2012), where sample thickness was 0.01 m and we estimated a leveling error of $\pm$ 0.05 m, a benchmark

229 error of $\pm$ 0.1 m and an angle of coring error that was 1% of depth. Collectively, these cores record the

230 time-dependent transgression of the site by late Holocene RSL rise

231

232   *3.4 Statistical analysis*

233 To objectively distinguish and describe regional microfossil groups using Partitioning Around Medoids

234 (PAM; e.g., Kaufman and Rousseeuw, 1990, Kemp et al., 2012a), we combined the results from all sites

235 into a single dataset of foraminifera and a single dataset of testate amoebae. Species counts were

236 expressed as percentages and samples that yielded zero or low counts were excluded prior to the analysis.

237 We determined the number of distinct assemblages within each dataset using the maximum average

238 silhouette width calculated for 2 to 20 groups. Silhouette widths close to 1 (maximum possible value)

239 indicate that a sample was classified appropriately, while values close to -1 (minimum possible value)

240 reflect poor classification. This metric aims to minimize within group variance, while maximizing

241 differences among groups (Rousseeuw, 1987). To investigate the ecological plausibility of our RSL

242 reconstructions, we measured the (Bray-Curtis) dissimilarity between each microfossil assemblage in the

243 core samples to their closest modern analog in the modern training sets of foraminifera and testate

244 amoebae. If the measured dissimilarity was less than the 20th percentile of dissimilarity measured among

245 all possible pairings of modern samples then we deemed the core sample to have an acceptable modern

246 analog (e.g., Jackson and Williams, 2004, Watcham et al., 2013). Core samples with a measured

247 minimum dissimilarity that exceeded this threshold were deemed to lack a modern analog.

248

249 To facilitate comparison among sites with different tidal ranges, we standardized elevation using a variant

250 of the standardized water level index (SWLI) of Horton (1999). We used MTL as the lower datum (SWLI

251 = 100) and the highest occurrence of foraminifera as the upper datum (SWLI = 200) because Wright et al.

252 (2011) demonstrated that this approach improved assemblage alignment in higher marsh settings where

253 the relationship between elevation and inundation is often nonlinear.

254

255 **4. RESULTS**

256 4.1 *Foraminifera*

257 The highest occurrence of foraminifera at Placentia was 0.75 m above MTL (PLA-B station 11) and

258 samples above this elevation on all three transects were devoid of foraminifera (Figure 2). Each of the

259 three sampled transects displayed a similar distribution of foraminifera. On transect PLA-A samples from

260 0.41–0.70 m were dominated by *Jadammina macrescens* and *Balticammina pseudomacrescens* (39-98%

261 when combined). *Trochammina inflata* was an important part of the assemblage (45-73%) at 0.22–0.46 m

262 MTL and *Miliammina fusca* characterized samples below 0.30 m MTL (more than 25–50%). On transect

263 PLA-B *Jadammina macrescens* and *Balticammina pseudomacrescens* were the dominant species above

264 0.69 m MTL (up to 37% and 62% respectively and up to 96% when combined), while *Trochammina*

265 *inflata* comprised 62-77% of individuals at 0.51–0.63 m MTL. *Miliammina fusca* was rare (<4%), but the

266 lowest sample on this transect was above MHHW. On transect PLA-C a single sample at 0.68 m MTL

267 yielded a foraminiferal assemblage that was 34% *Jadammina macrescens* and 64% *Balticammina*

268 *pseudomacrescens*. *Trochammina inflata* comprised 32–68% of individuals from 0.34 m to 0.55 m MTL.

269 Assemblages from 0.22 m to 0.43 m MTL included an average of 28% *Miliammina fusca*.

270 *Haplophragmoides* spp. were absent on all three transects at Placentia.

271

272 Foraminifera along two transects at Hynes Brook (HBM-0 and HBM-2; Table 1) were described by

273 Wright et al. (2011). Results from a third transect (HBM-1) described here for the first time show a very

274 similar distribution of foraminifera. The highest occurrence of foraminifera among all three transects was

275 at 0.90 m MTL (HBM-1 station 9; Figure 3), which is 0.05 m higher than estimated by Wright et al.

276 (2011) from the smaller dataset. On transect HBM-1 *Jadammina macrescens* (up to 63%) and

277 *Balticammina pseudomacrescens* (up to 83%) made up the majority of individuals (57–100% when

278 combined) at 0.50–0.90 m MTL, while *Miliammina fusca* dominated (44–86%) samples from 0.15 m to

279 0.45 m MTL. *Tiphotrocha comprimata* and *Haplophragmoides manilaensis* were present on HBM-1.

280 *Trochammina inflata* was absent or rare (maximum abundance of 7.8%) on the three transects from

281 Hynes Brook.

282

283 The distribution of foraminifera at Big River is largely consistent among transects and there is also a high

284 degree of similarity to those at Hynes Brook. The highest occurrence of foraminifera among all three

285 transects was 0.88 m MTL (BRM-2 station 7; Figure 3). On transect BRM-0 elevations above MHW

286 (0.31–0.86 m MTL) were dominated by *Jadammina macrescens* (up 93%) and *Balticammina*

287 *pseudomacrescens* (up to44%; 58-100% when combined), while *Miliammina fusca* was the characteristic

288 species (53-82%) in assemblages at -0.09 m to 0.26 m MTL. *Tiphotrocha comprimata* was present on this

289 transect (up to 39%). On transect BRM-1, assemblages of foraminifera from 0.10 m to 0.85 m MTL were

290 dominated by *Jadammina macrescens* (up to 40%) and *Balticammina pseudomacrescens* (up to 85%;

291 49-100% when combined). *Miliammina fusca* made up 31–66% of individuals at 0.05–0.20 m MTL. On

292 transect BRM-2 elevations from 0.33 m to 0.88 m MTL are characterized by high abundances of

293 *Jadammina macrescens* (up to 62%) and *Balticammina pseudomacrescens* (up to 60%; 41–86% when

294 combined). Two samples at 0.23 m and 0.28 m MTL are dominated by *Miliammina fusca* (81% and 66%

295 respectively). The abundance of *Haplophragmoides manilaensis* at Big River demonstrates variability

296 among transects (maximum abundance of <5% on BRM-0, but up to 49% on BRM-1 and 37% on

297 BRM-2) as does *Trochammina inflata* (maximum abundance of 29% on BRM-0, 2.5% of BRM-1 and 6%

298 on BRM-2), which may reflect a patchy distribution (e.g., Kemp et al., 2011, Swallow, 2000) and/or

299 within site variability of secondary environmental factors such as salinity or sediment texture that were

300 not measured. Modern foraminifera data are presented in the supporting appendix.

301

302   *4.2 Testate amoebae*

303 At Placentia the lowest occurrence of testate amoebae occurred below MHHW at 0.30 m MTL (PLA-A

304 station 23) with lower samples yielding fewer than 9 individuals after counting a sufficient volume of

305 material to recognize 100 *Lycopodium* spores (Figure 4). Samples above 0.63 m MTL were dominated by

306 *Tracheleuglypha dentata* (average 16%) and *Euglypha rotunda* type (average 26%). Because the highest

307 sample on the transect included abundant testate amoebae, the upper limit of this assemblage cannot be

308 estimated. At 0.30–0.55 m MTL the most abundant species was *Centropyxiella* type (average 33% and up

309 to 63%). Other common species on PLA-A included *Pseudocorythion* type (up to 30%) and

310 *Pseudohyalosphenia* type (up to 22%).

311

312 At Hynes Brook testate amoebae were sparse (fewer than 10 individuals per 100 *Lycopodium* spores) at

313 stations 10–15 and the lowest occurrence of testate amoebae was established at 0.70 m above MTL

314 (Figure 4), which is slightly above MHHW. At elevations above 0.98 m above MTL, the most common

315 species are *Trinema lineare* type (17–26%) and *Trinema enchelys* type (23–34%). The samples between

316 0.74 m MTL and 0.93 m MTL were characterized by an increased abundance of *Euglypha rotunda* type

317 (20% in each of the three samples). The lowest viable assemblage of testate amoebae includes more than

318 40% *Centropyxis cassis* type.

319

320 At Big River, the lowest occurrence of testate amoebae was recorded at 0.52 m above MTL (0.01 m

321 above MHHW) in the sample from BRM-0b station 11 (Figure 5). At elevations from 0.56 m to 1.51 m

322 MTL, assemblage composition resembles those identified at Hynes Brook, with the most abundant

323 species being *Trinema lineare* type (up to 25%), *Trinema enchelys* type (up to 30%), and *Euglypha*

324 *rotunda* type (up to 27%). The samples at 0.52 m and 0.57 m MTL are characterized by increased

325 abundance of *Centropyxis cassis* type (>34%) and the appearance of *Arcella catinus* (up to 35%) at the

326 lower limit of testate occurrence. Modern testate amoebae data are presented in the supporting appendix.

327

328   *4.3 Regional-scale microfossil distributions*

329 We combined the modern foraminifera results from Placentia, Hynes Brook and Big River into a single

330 dataset comprised of 134 samples (Figure 5). Analysis of this dataset using PAM indicates two plausible

331 ways to partition the samples. A maximum average silhouette width is returned by dividing the dataset

332 into two groups based on the relative abundance of *Miliammina fusca* versus *Jadammina macrescens* and

333 *Balticammina pseudomacrescens*. This pattern describes the universal distinction between low and high

334 salt-marsh samples. A more nuanced classification is possible by partitioning the regional-scale dataset

335 into five groups. Group 1 is dominated by *Jadammina macrescens* with correspondingly low occurrences

336 of *Balticammina pseudomacrescens*. This group is present at Hynes Brook and Big River, but is absent

337 from Placentia. Group 2 is dominated by *Jadammina macrescens* and *Balticammina pseudomacrescens*

338 and was present at all sites. Group 3 is a diverse high salt-marsh assemblage in which *Tiphotrocha*

339 *comprimata* and *Haplophragmoides manilaensis* occur alongside *Jadammina macrescens* and

340 *Balticammina pseudomacrescens*. This group was present at each of the three study sites. Group 4 is

341 comprised of low salt-marsh assemblages characterized by high abundances of *Miliammina fusca* and is

342 absent from Placentia due to the lack of a well-developed low salt-marsh environment at the site today.

343 Group 5 is dominated by samples from Placentia and captures the uniquely high relative abundances of

344 *Trochammina inflata* in these transects.

345

346 We combined the samples from Placentia, Hynes Brook and Big River to create a regional-scale dataset

347 of testate amoebae from 43 modern samples (Figure 5). Partitioning around medoids showed that the

348 maximum average silhouette width (0.38) occurred when the dataset was divided into two groups and no

349 finer-scale sub-division is warranted. Group 1 (30 samples) was represented by *Trinema lineare* type

350 (13%), *Trinema enchelys* type (18%) and *Euglypha rotunda* type (20%). This group was present at all

351 three sites. In contrast, Group 2 (13 samples) only occurred at Placentia and in a single sample from

352 Hynes Brook (HBM-0b station 11). It is characterized by *Arecella catinus* (discoides group),

353 *Centropyxiella* type (25%) and *Pseudohyalosphenia* type (Ooms; 22%).

354

355   *4.4 Bulk-sediment $\delta^{13}C$ values*

356 Despite differences in floral communities and microfossil assemblages, measured bulk-sediment $\delta^{13}C$

357 values from each of the three study sites exhibit no consistent trend with station elevation (Figures 2 & 5)

358 and largely fall in the range of –24‰ to –29‰ (data are provided in the supporting appendix). The

359 exceptions to this pattern are three stations from the middle of transect PLA-A at Placentia, which

360 returned values of –21‰ (the maximum typically associated with C_3 vegetation) and –15‰ to –17‰,

361 which is characteristic of C_4 terrestrial plants and also some marine algae (e.g., Lamb et al., 2006). At the

362 time of sampling these stations were vegetated predominantly by *Spartina patens* (a C_4 plant) and *Juncus*

363 *geradii* (a C_3 plant). Despite the presence of C_4 plant species on the salt marshes at each of our study sites

364 (e.g. *Spartina patens* and *Distichlis spicata*) the pervasive bulk-sedimentary signatures are those

365 reflecting C_3 plant material (Lamb et al., 2006).

366

367   *4.5 Radiocarbon dates and microfossil assemblages*

368 We collected three cores from Hynes Brook that were positioned to capture the transition from

369 coarse-grained clastic sediment to basal organic sediment at different elevations marking the

370 time-dependent transgression of RSL during the late Holocene (Figure 6). In core HBM-C102 (48.60133

371 °N; 58.94578 °W) a radiocarbon date from 2.11 m yielded a calibrated age of 2718–2489 years BP (2σ

372 range; Table 2). Foraminifera from the dated interval are comprised almost exclusively of *Jadammina*

373 *macrescens* and *Balticammina pseudomacrescens*. The most abundant testate amoebae in these samples

374 were *Centropyxis cassis* type (63–78%) and *Difflugla lucida* type (10–12%). In core HBM-C103

375 (48.60135 °N; 58.94575 °W) a radiocarbon date from 0.99 m yielded a calibrated age of 1825–1713 years

376 BP (2σ range; Table 2). Foraminifera from the dated interval are comprised almost exclusively of

377 *Jadammina macrescens* and *Balticammina pseudomacrescens*. The most abundant testate amoebae in

378 these samples were *Centropyxis cassis* type (64–62%) and *Centropyxis platystoma* type (14–34%). In

379 core HBM-C104 (48.60133 °N; 58.94584 °W) a radiocarbon date from 1.28 m yielded a calibrated age of

380 1736–1612 years BP (2σ range; Table 2). Foraminifera from the dated interval are 100% *Jadammina*

381 *macrescens* and *Balticammina pseudomacrescens*. The most abundant testate amoebae in these samples

382 were *Centropyxis cassis* (54–70%), *Centropyxis platystoma* (6–14%) and *Centropyxis delicatula-ecornis*

383 (up to 16%). In each core the consistency of microfossil assemblages indicates that no significant

384 environmental change took place over the period of time represented by the counted samples.

385 Bulk-sediment $\delta^{13}C$ measurements from all of the samples in each core yielded values of approximately –

386 26 ‰. All assemblages of foraminifera had good modern analogs, while all assemblages of testate

387 amoebae lacked modern analogs due to the high abundance of *Centropyxis cassis* in core samples (54–

388 78%) compared to the modern dataset (maximum of 43%).

389

390   **5. DISCUSSION**

391   *5.1 Distribution of salt-marsh foraminifera*

392 Some of the earliest investigations into the use of salt-marsh foraminifera as sea-level indicators were

393 conducted in Nova Scotia (Scott and Medioli, 1978, 1980), where intertidal transects revealed a

394 characteristic vertical zonation of distinct assemblages. Shallow sub-tidal environments included large

395 numbers of calcareous species that became scarce in low salt-marsh assemblages dominated by the

396 agglutinated species *Miliammina fusca*. High salt-marsh environments were dominated by *Jadammina*

397 *macrescens* with *Trochammina inflata* and *Tiphotrocha comprimata*. A monospecific zone of *Jadammina*

398 *macrescens* at the transition from salt marsh to upland occupied the narrowest range of elevation and was

399 consequently deemed to be the assemblage that could produce the most precise RSL reconstruction. Since

400 this work, the utility of salt-marsh (and mangrove) foraminifera as sea-level indicators has been

401 reinforced by recognition of vertically-zoned assemblages at sites around the world under a wide range of

402 climate, salinity and tidal conditions (e.g., Barbosa et al., 2005, Hayward et al., 1999, Horton and

403 Edwards, 2006, Horton et al., 2005, Leorri et al., 2008, Patterson et al., 2005, Scott et al., 1996, Spencer,

404 2000). However, the composition of high salt-marsh assemblages in particular varies among (and within)

405 regions, as do the specific elevation of boundaries between zones (e.g., Edwards and Wright, 2015, Kemp

406 et al., 2009, Wright et al., 2011). This pattern necessitates using an appropriate training set to provide

407 adequate modern analogs for interpreting assemblages preserved in the sedimentary record (e.g., Edwards

408 et al., 2004). Since surface and sub-surface assemblages at a site may differ due to environmental change

409 through time, the search for appropriate modern analogs commonly requires compilation of data from

410 multiple locations (e.g., Horton and Edwards, 2005). As part of this process, new insights into the ecology

411 of salt-marsh foraminifera can be gained that may, in turn, improve the quality of foraminifera-based RSL

412 reconstructions.

413

414 To establish the regional-scale relationship between assemblages of salt-marsh foraminifera and tidal

415 elevation in Newfoundland we identified five distinctive groups of foraminifera using PAM and by

416 standardizing sample elevations to account for differences in tidal range among sites (Figures 5 and 7).

417 The major faunal turnover occurs at ~140 SWLI and is characterized by a transition from a low

418 salt-marsh assemblage dominated by *Miliammina fusca* (Group 4; 86–140 SWLI) to spatially-variable

419   high salt-marsh assemblages in which *Jadammina macrescens* (Group 1; 134–200 SWLI), *Balticammina*

420   *pseudomacrescens* (Group 2; 133–200 SWLI), or *Trochammina inflata* (Group 5; 129–184 SWLI) is the

421   dominant species. Group 3 (111–187 SWLI) straddles the boundary between low and high salt-marsh

422   assemblages and is characterized by greater species diversity including the presence of *Tiphotrocha*

423   *comprimata* (all three sites) and *Haplophragmoides manilaensis* (absent at Placentia and rare in parts of

424   Big River).

425

426   Significantly, Groups 1 and 2 extend over the same vertical range and either may characterize the

427   uppermost salt-marsh samples. Consequently, when considered as tools for RSL reconstruction, the

428   distinction between *Jadammina macrescens* and *Balticammina pseudomacrescens* provides little or no

429   improvement of precision or accuracy over the 'lumping' of both species into a single taxon. In

430   Newfoundland, since no other groups extend to the upper limit of marine influence, relative abundances

431   of *Jadammina macrescens* and/or *Balticammina pseudomacrescens* greater than ~80% are indicative of

432   accumulation in the highest salt-marsh environment.

433

434   Group 5 is dominated by *Trochammina inflata* and (with the exception of a single sample at Big River) is

435   only present at Placentia, suggesting particular suitability to environmental conditions at that site. High

436   abundances of *Trochammina inflata* coincide with an absence or relative scarcity of *Balticammina*

437   *pseudomacrescens*, which may reflect differing environmental preferences or competitive exclusion.

438   Since all of our study sites share a similar atmospheric and oceanographic climate (Figure 1), it is

439   tempting to ascribe the observed distribution of Group 5 to a prevailing regime of lower salinity at

440   Placentia. The geomorphology at Placentia restricts direct exchange of water with the ocean and the site's

441   position in the south arm likely produces a water mass with lower salinity than at Hynes Brook and Big

442   River where there is direct access to ocean water and little fluvial input. Furthermore, the steep slopes at

443   the rear of the Placentia salt marsh cause freshwater to drain directly onto the marsh and at the time of

444   sample collection we observed several springs at the rear of the marsh that serve to further dilute the

445   salinity at higher tidal elevations. However, *Trochammina inflata* was not noted elsewhere as a

446   low-salinity indicator. In fact, *Balticammina pseudomacrescens* and *Haplophragmoides* spp. are more

447   typically linked to low-salinity conditions (de Rijk and Troelstra, 1997, Edwards and Wright, 2015).

448   Therefore further work is necessary to distinguish the relative importance of secondary environmental

449   variables as a cause of among-site and among-transect variability in foraminiferal assemblages.

450

451   The pattern of zonation that we observed at Hynes Brook and Placentia is similar to that described by

452   Daly (2002), who recognized three groups of foraminifera from a total of 29 surface sediment samples

453   collected at four Newfoundland salt marshes (Hynes Brook, St. Paul's Inlet, Village Cove and Placentia;

454   Figure 1). The three groups of Daly (2002) are all from high salt-marsh environments because sampling

455   did not extend below mean high water (MHW). The dominant species in these groups were *Jadammina*

456   *macrescens* with *Balticammina pseudomacrescens*, *Jadammina macrescens* and *Balticammina*

457   *pseudomacrescens*. Assemblages of salt-marsh foraminifera in Newfoundland closely resemble those

458   described at sites in the Canadian Maritimes including, Prince Edward Island (Scott et al., 1981), Nova

459   Scotia (Scott and Medioli, 1978, 1980, Smith et al., 1984), New Brunswick (Patterson et al., 2004) and

460   the Magdalen Islands (Barnett et al., 2016). A similar distribution is present in Maine, USA (Gehrels,

461   1994). We conclude that salt-marsh foraminifera from our three study sites (and others; e.g., Daly, 2002,

462   Daly et al., 2007) in Newfoundland conform to the pattern of vertical zonation observed in surrounding

463   regions and confirm that they are viable sea-level indicators in this region. A notable characteristic of the

464   foraminiferal assemblages from Newfoundland is their low species diversity (e.g., Daly, 2002; Figure 7),

465   which results in bipartite division of the salt marshes into near-monospecific high and low salt-marsh

466   assemblages. This pattern is also typical at other high latitude sites in the northern hemisphere that

467   experience a cold maritime climate such as arctic Norway, (Barnett et al., 2015), Iceland (Gehrels et al.,

468   2006b), the Aleutian Islands of Alaska (Kemp et al., 2013a), Denmark (Gehrels and Newman, 2004) and

469   the White Sea (Russia; Kemp et al., Submitted). These low-diversity assemblages stand in contrast to

470   those from warmer climates along the U.S. Atlantic coast where several studies reported a larger diversity

471   of species at single sites, but also marked variability in the composition of high salt-marsh assemblages

472   among sites (e.g., Goldstein and Frey, 1986, Kemp et al., 2009, Kemp et al., 2013d, Wright et al., 2011).

473   We contend that these differences among regions arise from the climate regime, where cold air and ocean

474   temperatures limit the diversity of high salt-marsh foraminiferal assemblages to a handful of species,

475   namely *Jadammina macrescens* and *Balticammina pseudomacrescens*. Additional systematic work is

476   needed to identify the specific ecological conditions (e.g. water temperature, seasonal air temperature,

477   frequency and duration of freezing) that drive this geographic gradient in species distributions. If it can be

478   adequately understood then temporal changes in salt-marsh foraminiferal assemblages may yield insight

479   into past climates as well as RSL change.

480

481   *5.2 Distribution of testate amoebae*

482   Intertidal testate amoebae were initially identified in Atlantic Canada (Medioli and Scott, 1983), often in

483   samples of salt-marsh sediment being analyzed for foraminifera (Scott et al., 1983, Scott, 1977, Scott and

484   Martini, 1982), but it was not until the late 1990s that intertidal environments were systematically

485   sampled for testate amoebae in order to establish their utility as sea-level indicators (Charman et al.,

486   1998). Subsequent studies demonstrated that the lowest occurrence of testate amoebae in salt marshes

15

487 typically occurs at, or slightly below, high tidal datums such as mean high water spring tide or MHHW at

488 mid-latitude sites in the northern hemisphere (Barnett et al., 2013, Gehrels et al., 2006a, Gehrels et al.,

489 2001). Similarly, the concentration of testate amoebae in surface sediment samples from Newfoundland

490 decreased abruptly near MHHW. Typical test concentrations above MHHW were ~2000 to ~5000 tests

491 per cm^3, but below MHHW counting statistically significant numbers of testate amoebae became

492 unviable. This pattern occurs because few taxa are tolerant of the increasingly brackish conditions

493 experienced below MHHW (Patterson and Kumar, 2002). Therefore the presence/absence of viable

494 testate amoebae populations is a useful paleo-ecological constraint for establishing if sediment samples

495 from a core accumulated above/below the lowest occurrence of testate amoebae that occurs close to

496 MHHW, but whose specific elevation must be established from an appropriate modern training set.

497

498 Using PAM, we identified two distinct groups of testate amoebae in Newfoundland (Figure 5, 7). Group 1

499 is characterized by high abundances of *Trinema enchelys* type, *Trinema lineare* type*, Euglypha rotunda*

500 type and *Centropyxis cassis* type (Figure 5). This group occurred at all sampled elevations at Hynes

501 Brook and Big River and in the uppermost samples at Placentia. Consequently, it spans a minimum

502 elevational range of 126–269 SWLI because its lower limit is constrained, but its upper limit is not. These

503 taxa also dominated similar environments from the nearby Magdalen Islands in the Gulf of St Lawrence

504 (Barnett et al., 2016) and are widespread in salt marshes throughout the North Atlantic (Barnett et al.,

505 2013, Charman et al., 2002, Gehrels et al., 2006a, Gehrels et al., 2001, Ooms et al., 2015), suggesting that

506 certain intertidal taxa may be cosmopolitan in and around salt marshes, although a synthesis of existing

507 studies, datasets and taxonomies would benefit the development of this proxy for future sea-level

508 research. Group 2 occurs at Placentia, but not Hynes Brook or Big River. It is comprised of

509 *Centropyxiella* type, *Pseudocorythion* type, *Pseudohyalosphenia* spp. and *Corythionella* type (Figure 5)

510 and exists at elevations from 140-187 SWLI (its upper and lower limits are constrained by the modern

511 dataset). *Centropyxiella* type was also common at comparable elevations in the Magdalen Islands

512 (Barnett et al., 2016), the UK (Charman et al., 2002) and Norway (Barnett et al., 2013), but sometimes

513 under different names because the nomenclature for salt-marsh testate amoebae is based on a wide

514 diversity of literature and has evolved over recent decades. Differences in the lowest occurrence of testate

515 amoebae and assemblage composition between sites in Newfoundland probably occurs in response to

516 secondary environmental variables such as salinity, pH and sediment composition (Barnett et al., 2016,

517 Charman et al., 2002). The geomorphological setting and freshwater input to the marsh at Placentia is the

518 most likely cause of the differences between sites. Lower salinity as a result of runoff and reduced

519 penetration of ocean waters at Placentia would provide favorable conditions for testate amoebae at lower

520   elevations than at the other sites. Increased runoff can also be an influence on pH and sediment substrate

521   that may be additional factors influencing the testate amoebae assemblages (e.g., Charman et al., 2002).

522

523   *5.3 Bulk-sediment $\delta^{13}C$ values as a sea-level indicator*

524   Salt-marsh plants are sea-level indicators because the varied tolerance of species to the frequency and

525   duration of tidal inundation results in a systematic pattern of zonation, where low and high salt-marsh

526   communities at the same site are vegetated by different species (e.g., Eleuterius and Eleuterius, 1979,

527   Johnson and York, 1915, Redfield, 1972). However, the composition of these zones can vary among sites

528   as a result of secondary environmental factors such as climate and salinity. On the U.S. Atlantic coast for

529   example, high salt-marsh environments are typically vegetated by *Spartina patens* and *Distichlis spicata*

530   at locations north of Chesapeake Bay, while *Juncus roemerianus* occupies this ecological position at

531   locations to the south (Eleuterius, 1976), until mangroves replace salt marshes in southern Florida (Figure

532   1).

533

534   As an adaptation to regular submergence, some salt-marsh plants (e.g., *Spartina* spp., *Distichlis spicata*)

535   utilize the C_4 (Hatch-Slack) photosynthetic pathway in which fractionation against atmospheric $^{13}CO_2$ in

536   favor of $^{12}CO_2$ is less pronounced than in C_3 (Calvin-Benson) plants such as *Juncus* spp. and the majority

537   of terrestrial species that live above the highest reach of tides. As a result, $\delta^{13}C$ values measured in C_4

538   plants are usually less depleted/negative than –17‰ compared to the VPDB standard, while C_3 plants

539   return more depleted/negative values between –21‰ and –32‰ (e.g., Lamb et al., 2006). In the organic

540   salt-marshes of eastern North America, the primary source of sediment is the *in situ* accumulation of

541   above and below ground biomass from the surface plant community and bulk sediment $\delta^{13}C$ values reflect

542   the dominant plant community at the time of deposition (e.g., Chmura and Aharon, 1995).

543   Post-depositional processes (principally preferential biodegradation of cellulose over lignin) cause

544   bulk-sediment $\delta^{13}C$ values to differ slightly, but systematically, from the parent plant material (e.g.,

545   Benner et al., 1991, Benner et al., 1987, Ember et al., 1987, Haines, 1976), although this change is not

546   large enough to prevent distinction between C_3 and C_4 plants. The use of bulk-sediment $\delta^{13}C$ values as a

547   sea-level indicator is reliant upon salt-marsh environments being dominated by C_4 plants such as *Spartina*

548   *patens* and *Spartina alterniflora*, which enables them to be readily and objectively distinguished from the

549   surrounding C_3-dominated freshwater upland environments. For example, peat-forming communities

550   between MTL and MHHW in New Jersey are vegetated almost exclusively by *Spartina* spp. and

551   *Distichlis spicata* resulting in bulk-sediment $\delta^{13}C$ values that are less depleted than –18.9‰, while

552   elevations above MHHW (including those without marine influence) had bulk-sediment $\delta^{13}C$ values more

553   depleted than –22‰ (Kemp et al., 2012b). However, sediment more depleted than –22‰ in which

554   foraminifera are present formed between MHHW and the highest occurrence of foraminifera. This pattern

555   enabled Kemp et al. (2013b) to reconstruct RSL using bulk-sediment $\delta^{13}C$ values, in which the precision

556   of a multi-proxy approach employing foraminifera and $\delta^{13}C$ values was up to 60% better than using

557   foraminifera alone (Cahill et al., 2016). In contrast, a similar study in North Carolina concluded that

558   bulk-sediment $\delta^{13}C$ values could not be objectively used as a sea-level indicator because the monospecific

559   high salt-marsh zone is vegetated by the C_3 species *Juncus roemerianus*, making it indistinguishable from

560   a freshwater upland (Kemp et al., 2010).

561

562   In Newfoundland bulk-sediment $\delta^{13}C$ values cannot objectively distinguish between material deposited in

563   a salt-marsh environment and material from the surrounding freshwater uplands that does not have a

564   systematic relationship to tidal datums (Figures 2 and 4). We therefore conclude that bulk-sediment $\delta^{13}C$

565   values have no utility as sea-level indicators in Newfoundland. Although this is a negative finding for the

566   potential to reconstruct RSL in our study region, it does help to refine the geographic range of locations

567   where bulk-sediment $\delta^{13}C$ values could be utilized. Eleuterius (1976) found that 49–77% of salt-marsh

568   area in North Carolina was vegetated by *Juncus roemerianus* (C_3 species) compared to <10% in Virginia

569   and Maryland and <0.1% in Delaware, where the high salt-marsh zone is almost exclusively comprised of

570   *Spartina patens*, *Spartina alterniflora* (short form) and *Distichlis spicata* (all C_4 species). Therefore we

571   propose that the southern limit for using bulk-sediment $\delta^{13}C$ values as a sea-level indicator on the Atlantic

572   coast of North America is close to Chesapeake Bay (Figure 1). On the U.S. Atlantic coast most high

573   salt-marsh environments in New England are solely vegetated by C_4 plants (e.g., Niering and Warren,

574   1980, Redfield, 1972). Although *Juncus geradii* becomes a common occurrence in Maine (e.g., Gehrels,

575   1994, Johnson et al., 2007) and southern Nova Scotia (e.g., Gordon Jr et al., 1985, Pielou and Routledge,

576   1976, Scott and Medioli, 1980), large areas of high salt marsh are comprised of *Spartina patens*

577   meadows, suggesting that bulk-sediment $\delta^{13}C$ values are likely to remain useful sea-level indicators in

578   this region. The increased floral diversity of high salt marshes in New Brunswick (e.g., Gehrels et al.,

579   2006a, Magenheimer et al., 1996), Prince Edward Island (e.g., Scott et al., 1981) and Newfoundland (e.g.,

580   Brookes et al., 1985; this study) results in a mosaic structure with increasing contributions to the surface

581   sediment from C_3 species such as *Juncus* spp., *Triglochin maritima* and *Plantago maritima*. Despite the

582   observed presence of *Spartina patens* and *Distichlis spicata*, we conclude that this diversity and structure

583   results in bulk-sediment $\delta^{13}C$ values which are characteristic of C_3 plants. Occasional samples with less

584   depleted values (e.g., stations 16 and 17 on PLA-A) likely represent persistent, but patchy stands of C_4

585   plants. Therefore the utility of bulk-sediment $\delta^{13}C$ values is restricted on the Atlantic coast of North

586   America to regions from Chesapeake Bay to southern Nova Scotia (Figure 1), although it is necessary to

587   recognize that geographic migration of floral zones may have occurred in response to Holocene climate

588   changes, which should be considered when choosing whether or not to measure $\delta^{13}C$ in core material.

589

590   *5.4 Reconstructing late Holocene relative sea level in Newfoundland*

591   We reconstructed RSL at Hynes Brook from three cores of basal sediment by combining the

592   radiocarbon-dated age of each sample with an estimate of paleo tidal elevation inferred from microfossil

593   assemblages preserved in core samples (Figure 8). This approach generated one sea-level index point

594   from each core. Paleo-tidal elevation was estimated from the combined presence of high salt-marsh

595   foraminifera (*Jadammina macrescens* and *Balticammina pseudomacrescens*) and testate amoebae. Since

596   the assemblages of testate amoebae lack modern analogs, we conservatively estimate that the dated

597   samples formed between the lowest occurrence of testate amoebae (0.52 m MTL at Hynes Brook) and the

598   highest occurrence of foraminifera (0.90 m MTL at Hynes Brook). Each dated sample came from within 5

599   cm of the basal contact and therefore experienced no, or minimal, post-depositional lowering due to

600   sediment compaction (Horton and Shennan, 2009).

601

602   The three new sea-level index points were combined with existing and standardized data from southwest

603   Newfoundland (Love et al., 2016; Figure 8). There is good agreement between these datasets, which

604   indicate that RSL rose in this region from approximately –3.5 m at 2900 years BP to present. The average

605   vertical and chronological uncertainty is smaller for the three new sea-level index points than for those in

606   the existing database. This pattern occurs because the age of our samples was established by radiocarbon

607   dating of single macrofossils, while some of the existing dates relied on thick sections of bulk sediment

608   that inherently included material spanning a range of ages. The vertical uncertainty in some of the

609   sea-level index points from the database is large because information provided in the original publication

610   was lacking and the data were standardized in a conservative manner (e.g., Engelhart et al., 2011,

611   Shennan and Horton, 2002). However, our results indicate that a simple multi-proxy approach using the

612   presence of high salt-marsh foraminifera and testate amoebae can produce precise RSL reconstructions.

613

614   The ecological plausibility of any paleoenvironmental reconstruction generated through reasoning by

615   analogy relies on their being an appropriate degree of similarity between modern and fossil assemblages

616   (e.g., Jackson and Williams, 2004). A long-running debate surrounding the use of microfossil groups to

617   reconstruct RSL is whether to use a local- or regional-scale modern training set to quantitatively interpret

618   assemblages preserved core samples (e.g., Horton and Edwards, 2005, Kemp and Telford, 2015,

619   Watcham et al., 2013). Local-scale datasets typically produce more precise reconstructions since the

620   effect of secondary environmental variables is minimized, while regional-scale datasets provide a broader

621   suite of analogs for generating reconstructions from sites where environmental conditions in the past may

622   not be the same as those today. The distribution of foraminifera in Newfoundland highlights the necessity

623   of compiling a regional-scale training set where the goal is to capture the principal surface assemblages

624   that are present on salt marshes in the region. For example, Placentia possess distinctive microfossil

625   assemblages (foraminifera Group 5 and testate amoebae Group 2) that are absent from Hynes Brook and

626   Big River. Although this diversity of modern assemblages was not necessary to accurately reconstruct

627   RSL from the Hynes Brook cores, it is likely that investigations spanning longer periods of time (and/or

628   from other sites) will require that a regional-scale training set be available. We conclude that a

629   regional-scale training set is necessary to capture the range of assemblages of foraminifera and testate

630   amoebae that are present on modern Newfoundland salt marshes.

631

632   All assemblages of testate amoebae in the three cores from Hynes Brook lacked modern analogs (Figure

633   6). The broad, biogeographical similarities between modern intertidal assemblages found in

634   Newfoundland (this study) and elsewhere in North America (Barnett et al., 2016, Gehrels et al., 2006a)

635   and Europe (Barnett et al., 2013, Charman et al., 2002, Ooms et al., 2015) suggests that this dissimilarity

636   between modern and fossil assemblages did not arise from temporal changes in the testate amoebae

637   population at the study site, unless ecological conditions differed so significantly in the past that Hynes

638   Brook supported an assemblage that is yet to be observed in modern North Atlantic salt marshes.

639   Alternatively, the lack of analogy between core and modern samples was caused by preferential

640   preservation where certain taxa are more or less likely to be lost through time due to differences in test

641   composition. This hypothesis is supported by comparison of the contemporary and fossil assemblages

642   from Newfoundland (Figure 9). The modern data contains an abundance of idiosomic genera (tests

643   composed of proteinaceous secretion and siliceous plates) such as *Arcella*, *Euglypha*, *Pseudocorythion*,

644   *Pseudohyalosphenia*, *Tracheleuglypha* and *Trinema*. In contrast, these genera are rarely encountered in

645   the fossil samples, where xenosomic genera (tests formed from agglutinated particles) represent the vast

646   majority of taxa (e.g., *Centropyxis*, *Cyclopyxis* and *Difflugia*). Ratios of idiosomic : xenosomic tests are

647   consistently two or three orders of magnitude greater in the surface assemblages than in fossil

648   assemblages (Figure 9), suggesting that idiosomic taxa were preferentially lost over time as evidenced

649   (for example) by the uncharacteristically extreme dominance of *Centropyxsis cassis* type (xenosomic test)

650   in the core samples. Analyses of coastal sediments from the UK reported good preservation of testate

651   amoebae in supratidal deposits (e.g., raised bogs and lakes), but poor preservation in mid-Holocene

652   salt-marsh sediments, including a lack of idiosomic genera (Lloyd, 2000, Roe et al., 2002). In contrast,

653   two short (~100 year) cores of salt-marsh sediment collected in Maine and Nova Scotia to reconstruct

654   RSL contained preserved idiosomic genera such as *Arcella*, *Euglypha, Tracheleuglypha* and *Trinema*

655   (Charman et al., 2010). Conditions in organic salt marshes (e.g., dissolved oxygen concentrations and pH)

656   are likely to promote loss of idiosomic tests (e.g. through dissolution). For example, Swindles and Roe

657   (2007) demonstrated experimentally that idiosomic tests were preferentially removed from testate

658   amoebae assemblages in low pH environments. While idiosomic tests may be preserved for several

659   hundred years, they can decay over longer time periods. Systematic studies that encompass a

660   comprehensive range of salt marsh and estuarine environments will greatly elucidate the viability of

661   testate amoebae as sea-level indicators from a preservation potential perspective. However, the simple

662   classification of presence or absence used in this study is unlikely to be unduly influenced by preservation

663   bias between idisomic and xenosomic tests.

664

665   **6. CONCLUSIONS**

666   Newfoundland is one of the most northern sites at which cores of salt-marsh sediment can yield detailed

667   RSL reconstructions to investigate physical processes that vary by latitude such as ocean circulation

668   changes (e.g., Levermann et al., 2005) and the fingerprint of Greenland Ice Sheet melt (Mitrovica et al.,

669   2011). In support of this work we investigated the utility of foraminifera, testate amoebae and

670   bulk-sediment $\delta^{13}C$ values as sea-level indicators in Newfoundland using modern (surface) sediment

671   samples collected along intertidal transects at Placentia (eastern Newfoundland) and Hynes Brook/Big

672   River (western Newfoundland).

673

674   Foraminifera are divided into five distinct groups which primarily discriminate low salt-marsh

675   environments dominated by *Miliammina fusca* (Group 4) from more diverse high salt-marsh zones with

676   assemblages dominated by *Jadammina macrescens* (Group 1), *Balticammina pseudomacrescens* (Group

677   2), or *Trochammina inflata* (Group 5). Group 3 spans the transition between the high and low marsh

678   groupings and comprises variable abundances of the secondary salt marsh taxa *Tiphotrocha comprimata*

679   and *Haplophragmoides manilaensis*. Group 5 is only present at Placentia, indicating the potential need to

680   compile regional-scale modern training sets to capture natural variability within a study region and

681   maximize the analogy between modern and fossil assemblages. Overall, the distribution of foraminifera

682   that we observed is similar to previous results from Newfoundland (e.g., Daly, 2002) and more generally

683   to other sites in the Canadian Maritimes and other high-latitude regions.

684

685   The lowest occurrence of testate amoebae at Hynes Brook and Big River occurs close to MHHW, but is

686   slightly lower at Placentia reflecting local environmental or taphonomic influences. Idiosomic taxa (e.g.,

687   *Trinema* spp., *Tracheleuglypha dentate* type, and *Euglypha* spp.), which characterize upper salt-marsh

688   environments along the east coast of North America were abundant in Newfoundland. However, the

689 difference in the ratio of idisomic and xenomic tests between modern and fossil samples suggests

690 assemblages of testate amoebae in salt marshes experience preservation bias in which idisomic tests are

691 preferentially removed. This preservation bias results in a lack of analogy between modern and fossil

692 assemblages.

693

694 Bulk-sediment $\delta^{13}C$ values cannot be used as sea-level indicators in Newfoundand because salt marsh and

695 freshwater upland environments yield values that are characteristic of C_3 plants and that cannot be

696 objectively distinguished from one another. We propose that the utility of this proxy for reconstructing

697 RSL in eastern North America is restricted to the coastline between Chesapeake Bay and southern Nova

698 Scotia.

699

700 We further tested the utility of these sea-level indicators by applying them to three radiocarbon-dated

701 cores of basal sediment from Hynes Brook. A simple, multi-proxy classification approach recognized this

702 sediment as having been deposited between the lowest occurrence of testate amoebae and the highest

703 occurrence of foraminifera. This interpretation is not effected by possible preservation bias of testate

704 amoebae in salt marshes. The three new sea-level index points are in agreement with those in an existing

705 database, but have smaller vertical (± 0.22 m) and temporal uncertainties ($\sim\pm 80$ years).

706

719   **Table 1**: Details of modern transects used to characterize the distribution of foraminifera, testate and

720   bulk-sediment δ13C values in Newfoundland.

Site	Transect	Year	Analysis	No. S	Range (m MTL)	Reference
Placentia	PLA-A	2015	F, T, δ^{13}C	25	0.30 to 1.22	This study
Placentia	PLA-B	2015	F, δ^{13}C	15	0.51 to 1.26	This study
Placentia	PLA-C	2015	F, δ^{13}C	10	0.23 to 1.21	This study
Hynes Brook	HBM-0	1999	F	25	–0.13 to 0.83	Wright et al. (2011)
Hynes Brook	HBM-0b	2015	T, δ^{13}C	15	0.24 to 2.16	This study
Hynes Brook	HBM-1	2001	F	24	0.15 to 1.28	This study
Hynes Brook	HBM-2	2001	F	22	0.12 to 1.15	Wright et al. (2011)
Big River	BRM-0	1999	F	25	–0.14 to 1.04	This study
Big River	BRM-1	2001	F	25	0.05 to 1.23	This study
Big River	BRM-1b	2015	T, δ^{13}C	13	0.42 to 1.51	This study
Big River	BRM-2	2001	F	20	0.23 to 1.16	This study

721

722   F = samples analyzed for foraminifera; T = samples analyzed for testate amoebae; δ^{13}C = samples

723   analyzed for bulk-sediment δ^{13}C values. No. S = total number of samples on transect (including those in

724   which foraminifera were absent).

725 **Table 2:** Radiocarbon ages and relative sea level (RSL) reconstruction from three cores of basal organic

726 sediment at Hynes Brook.

Core	Sample Elevation (m, MTL)	Sample ID	^{14}C Age	^{14}C Error	Indicative Meaning	Sampling Error (m)	RSL (m)
HBM C102	−1.38	OS-123524	2,490	20	LOT-HOF	0.17	−2.09 ± 0.22
HBM C103	−0.25	OS-123656	1,840	20	LOT-HOF	0.17	−0.96 ± 0.22
HBM C104	−0.55	OS-123525	1,770	20	LOT-HOF	0.17	−1.26 ± 0.22

727

728 Sample ID is the unique identifier assigned to each reported radiocarbon age by the National Ocean

729 Sciences Accelerator Mass Spectrometry facility. Indicative meaning is the tidal elevation at which each

730 sample was judged to have formed at based on the presence of both foraminifera (HOF = highest

731 occurrence of foraminifera) and testate amoebae (LOT = lowest occurrence of testate amoebae) in and

732 around the dated sample. At Hynes Brook and Big River, HOF is at 0.90 m MTL and LOT is at 0.52 m

733 MTL, resulting in a reference water level of 0.71 m above mean tide level and an indicative range of ±

734 0.19 m. The sampling error for each sea-level index point is the sum of estimated uncertainties for

735 leveling (± 0.05 m), benchmarks (± 0.1 m), sample thickness (± 0.01 m) and core angle (1% of depth

736 equating to ± 0.01 m for these samples). The RSL error was calculated following Engelhart and Horton

737 (2012).

738

739   **FIGURE CAPTIONS**

740

741   **Figure 1:** Location of study sites in Newfoundland, Canada. **(A)** Approximate geographic boundaries

742   between regions on the Atlantic coast of North America where high salt-marsh environments are

743   vegetated predominately by C_3 (e.g. *Juncus* spp.) and C_4 species (e.g. *Spartina patens*, *Distichlis spicata*),

744   where salt marshes replace mangroves and regions experiencing uplift or subsidence as a consequence of

745   ongoing glacio-isostatic adjustment. Number sites are locations discussed in text (Daly, 2002; 1 = St.

746   Paul's Inlet, 2 = Village Cove) **(B-D)** Study site at Placentia, close to the Argentia tide gauge. The site

747   experiences tidal inundation because the open North Arm is connected to the South Arm at the eastern

748   edge of the Placentia peninsula. The gravel barrier in front of the salt marsh is overwashed by high tides.

749   **(E-H)** Study sites at Hynes Brook and Big River. The location of the Port aux Basques tide gauge is

750   shown. Foraminifera from transects HBM-0 and HBM-2 were originally presented by Wright et al.

751   (2011). Three cores of basal sediment were collected along HBM-2. **(I)** Monthly average high (H) and

752   low (L) air temperature at Stephenville (1942–2014) and Argentia (1945–2007), calculated from

753   Government of Canada historic climate data.

754

755   **Figure 2:** Distribution of foraminifera and $\delta^{13}C$ values measured in samples of modern salt-marsh

756   sediment collected along three transects at Placentia, Newfoundland. HOF = highest occurrence of

757   foraminifera, which is the single highest sample from all three modern transects at Placentia to provide a

758   robust and *in situ* assemblage of foraminifera. MHHW = mean higher high water; MHW = mean high

759   water; MTL = mean tide level. Measured, bulk-sediment $\delta^{13}C$ values are expressed relative to the Vienna

760   Pee Dee Belemnite (VPDB) standard. Shaded intervals represent values that are typical of salt-marsh

761   sediment from floral zones dominated by C_3 and C_4 plant species.

762

763   **Figure 3:** Distribution of foraminifera in samples of modern salt-marsh sediment collected along three

764   transects at Big River and one transect at Hynes Brook in Newfoundland. Foraminifera along two other

765   transects at Hynes Brook (HBM-T0 and HBM-T2) were published by Wright et al. (2011) and are used in

766   our regional compilation of data, but are not presented in detail here. HOF = highest occurrence of

767   foraminifera, which is the single highest sample from the six modern transects at Big River and Hynes

768   Brook to provide a robust and *in situ* assemblage of foraminifera. MHHW = mean higher high water;

769   MHW = mean high water; MTL = mean tide level.

770

771   **Figure 4:** Distribution of testate amoebae in samples of modern salt-marsh sediment collected along

772   transect HBM-0b at Hynes Brook, BRM-1b at Big River and PLA-A at Placentia. Panels in the top row

25

773   show the position of sampling stations along each transect; note that the elevation axes differ among sites.

774   HOF = highest occurrence of foraminifera; LOT = lowest occurrence of testate amoebae (the sample

775   below which test abundance was insufficient to represent a viable and *in-situ* assemblage); MHHW =

776   mean higher high water; MTL = mean tide level. The concentration of testate amoebae per counted spore

777   of the exotic pollen *Lycopodium clavatum* (row 2) was used to determine LOT. Typically there was an

778   easily recognizable and abrupt decrease in test concentration at this point from ~0.2 to <0.1. Rows 3–6

779   show the distribution of the five most abundant species of testate amoebae on each transect, note that the

780   abundance axes are the same within each transect, but differ among them. The lowest row of panels

781   presents measurements of bulk-sediment δ^{13}C values. The profile for Placentia is presented in Figure 2.

782

783   **Figure 5:** Regional-scale modern datasets of foraminifera and testate amoebae from Hynes Brook, Big

784   River and Placentia in Newfoundland, Canada. **(A)** Average silhouette width calculated by partitioning

785   around medoids of the foraminifera dataset from which we recognized five distinct groups. **(B)**

786   Foraminifera dataset divided into five groups and colored by site. Hm = *Haplophragmoides manilaensis*;

787   Tc = *Tiphotrocha comprimata*. **(C)** Average silhouette width calculated by partitioning around medoids

788   of the testate amoebae dataset. These results demonstrated that two distinct groups should be recognized.

789   **(D)** Testate amoebae dataset divided into two groups and colored by site. Po = *Pseudohyalosphenia* type

790   (Ooms).

791

792   **Figure 6:** Samples of basal salt-marsh sediment from Hynes Brook that were analyzed to reconstruct late

793   Holocene relative sea-level. For each of the three cores (C102, top row; C103, middle row; C104 bottom

794   row), a lithology column shows the position of the basal contact and radiocarbon date with its unique

795   identifier from the National Ocean Sciences Accelerator Mass Spectrometry facility. Core top elevations

796   with respect to modern, local mean tide level (MTL) are listed. Abundance of *Jadammina macrescens*

797   (Jm) and *Balticammina pesudomacrescens* (Bp) are shown in the first column of panels (blue bars).

798   Dissimilarity between each core sample and its closest modern analog based on foraminifera are shown in

799   the second column of panels (dashed lines represent values for percentiles of dissimilarity measured in

800   pairings of modern samples). Abundance of the two most common species of testate amoebae are shown

801   in the third and fourth columns (red bars; DL= *Difflugia lucida* type; CP = *Centropyxis platystoma* type;

802   CD = *Centropyxis delicatula-ecornis* type). Note that the second most abundant species (after *Centropyxis*

803   *cassis* type) varies among cores. The concentration of testate amoebae is expressed relative to the

804   frequency of *Lycopodium* spores in the fifth column of panels. The dashed line represents the lowest

805   concentration in a modern sample that was considered to have a viable and *in-situ* assemblage. Values

806   exceeding this threshold indicate deposition above the lowest occurrence of testate amoebae.

807   Dissimilarity between each core sample and its closest modern analog based on testate amoebae are

808   shown in the fifth column of panels (dashed lines represent values for percentiles of dissimilarity

809   measured in pairings of modern samples). Measured, bulk-sediment $\delta^{13}C$ values with respect to the

810   Vienna Pee Dee Belemnite (VPDB) standard are presented in the final column and the shaded regions

811   denote the approximate range of C_3 and C_4 plants.

812

813   **Figure 7:** Relationship between species of modern **(A)** foraminifera and **(B)** testate amoebae and tidal

814   elevation in Newfoundland. Symbol color denotes group membership established by partitioning around

815   medoids applied separately to the regional modern datasets of each microfossil group. Colored bars at

816   right show the range of elevations over which each group was found. Arrows indicate groups for which

817   upper and/or lower limits could not be reliably established. Note that an anomalously low sample from

818   foraminifera group 5 was discounted in estimating the range of this group. Elevation is expressed as a

819   standardized water level index (SWLI), where a value of 200 is the highest occurrence of foraminifera

820   and 100 is mean tide level.

821

822   **Figure 8:** Late Holocene relative sea-level history of southwestern Newfoundland produced using a

823   standardized database of sea-level index points (Love et al., 2016; open rectangles) and the three new

824   basal sea-level index points from Hynes Brook (this study; filled rectangles labeled to denote the core

825   from which each new index point was produced). The study of Love et al. (2016) utilized several earlier

826   datasets to produce standardized sea-level index points (see references therein).

827

828   **Figure 9:** Ratio of testate amoebae with idiosomic (composed of proteinaceous secretion and siliceous

829   plates) and xenosomic (formed from agglutinated particles) tests in modern and fossil sediment samples

830   in Newfoundland. Bars represent modern samples (colored by site), while the blue, shaded region

831   represents the range of values encountered in core samples from Hynes Brook. The apparent loss of

832   idiosomic tests is interpreted as preservation bias.

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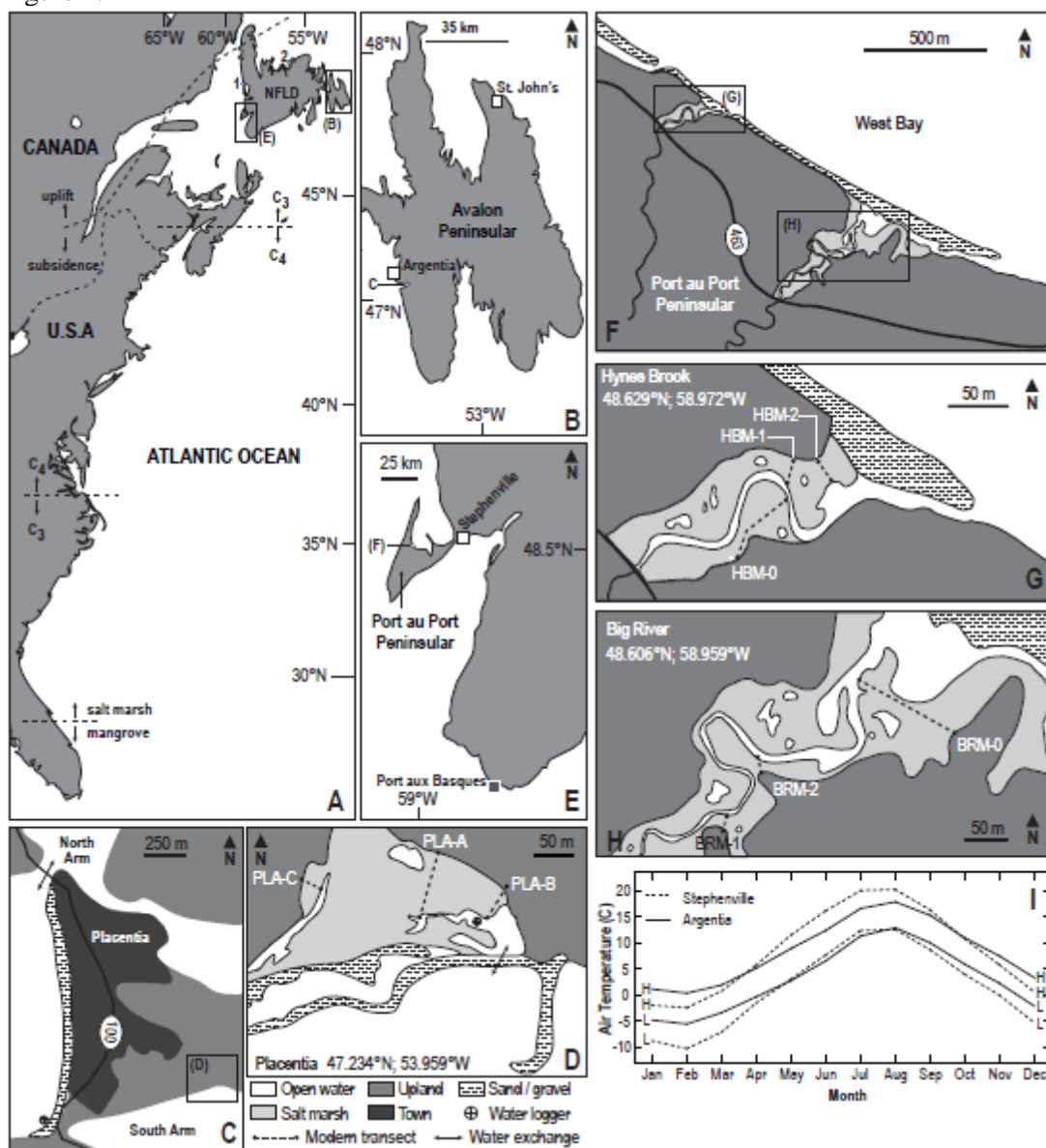977 from New Jersey, USA. Quaternary Science Reviews 54, 26-39.

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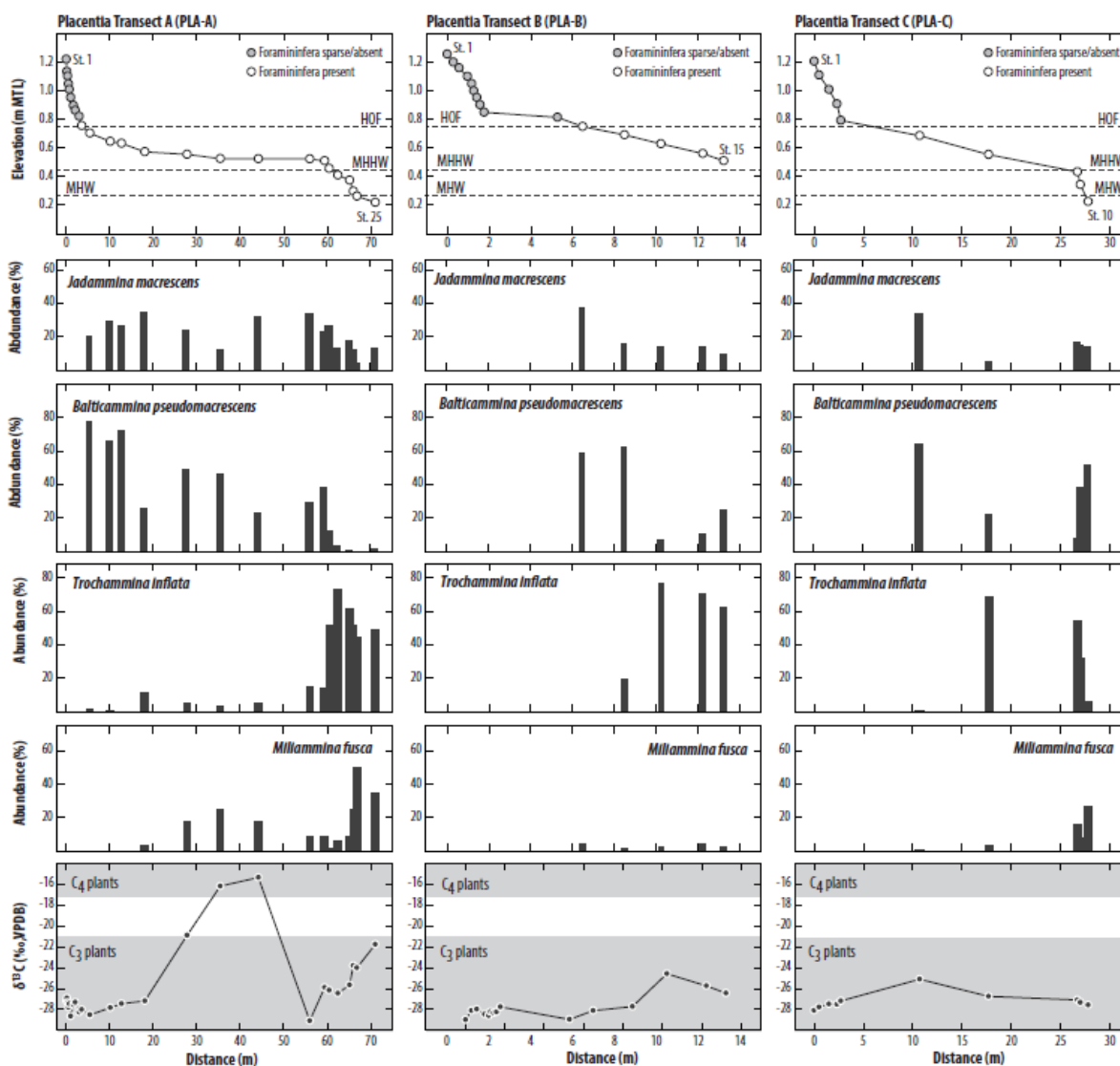1085   States. Geophysical Research Letters 40, 5514-5520.

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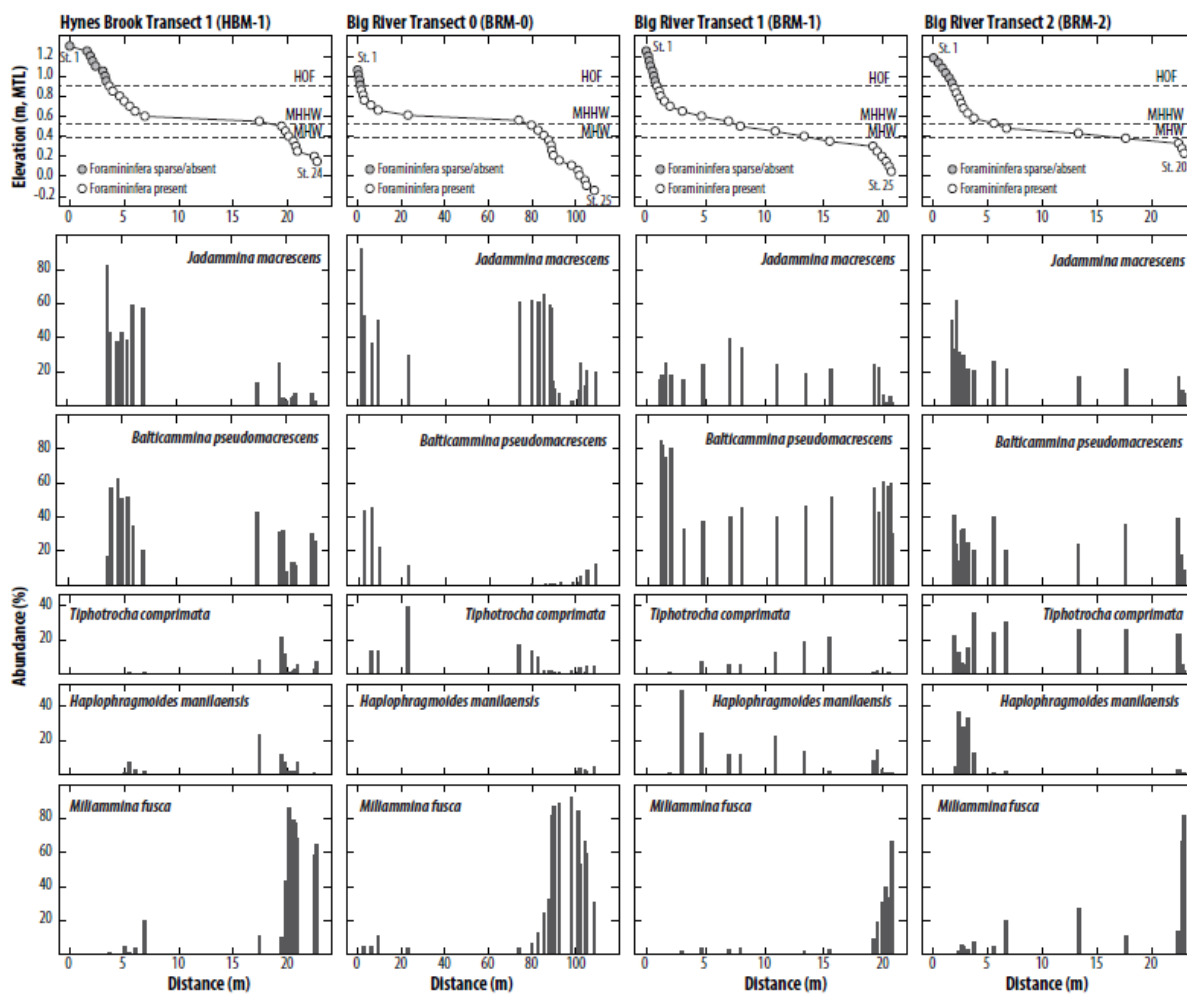1089   Level Research. Wiley, pp. 233-248.
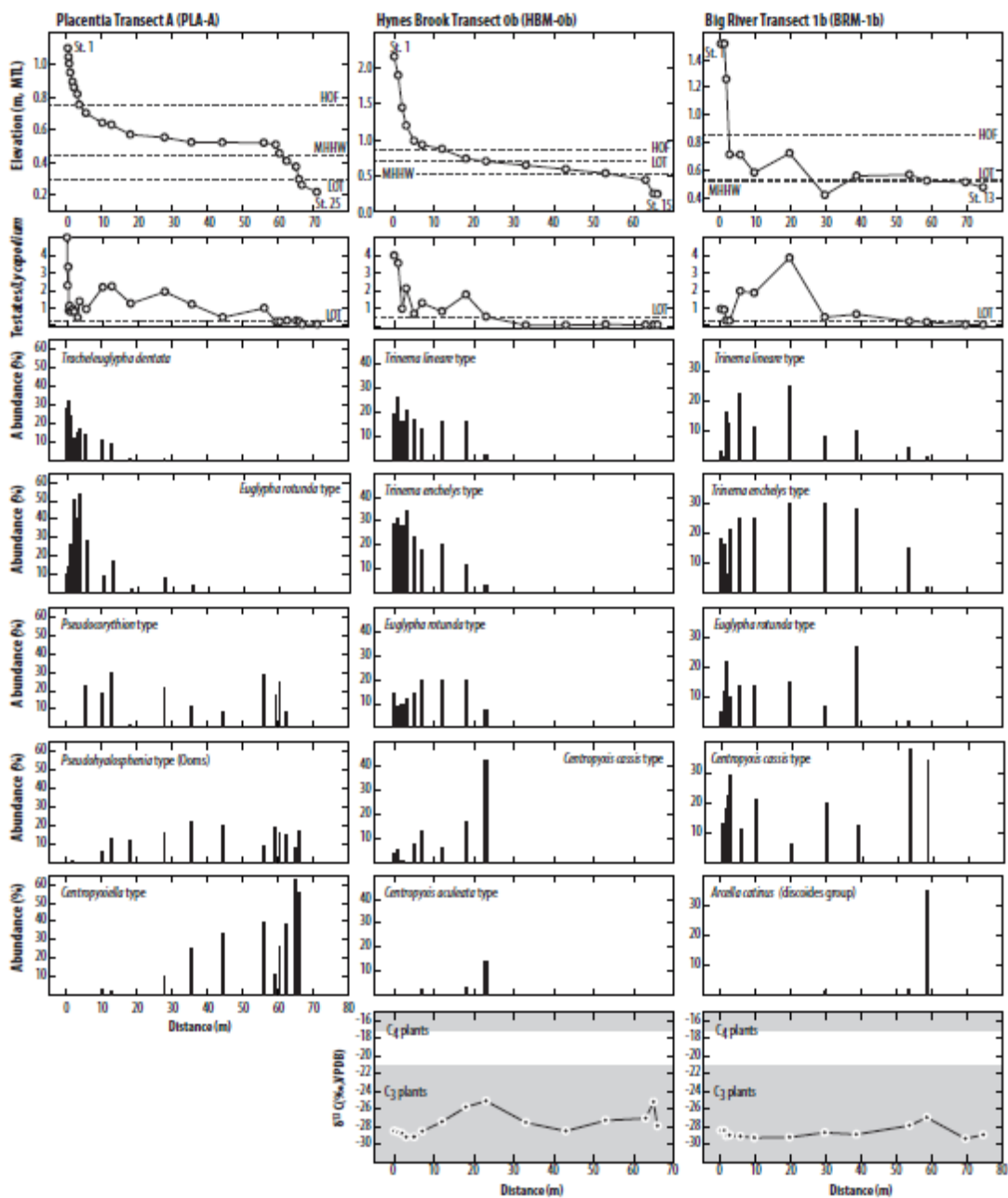1090

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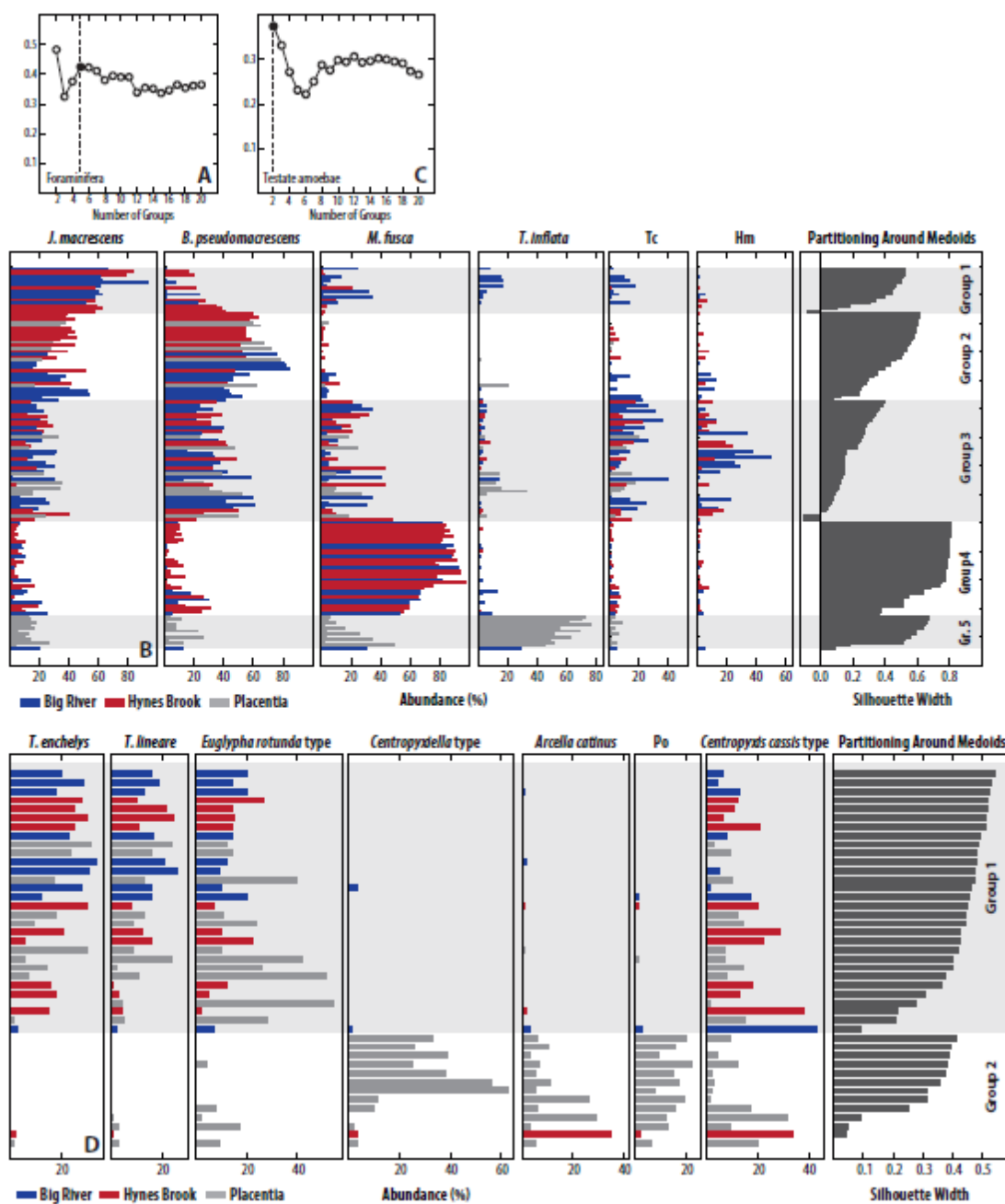1092
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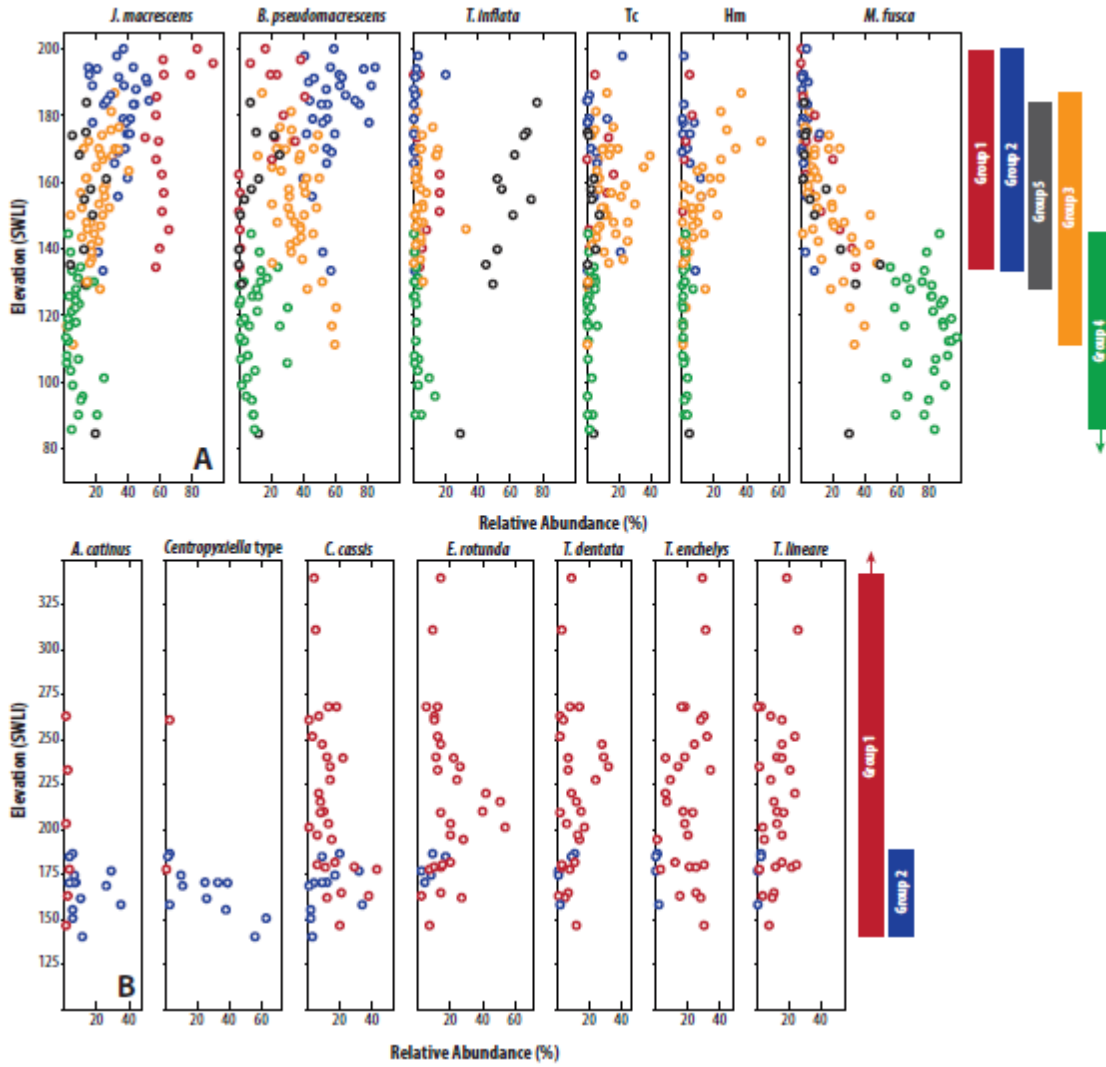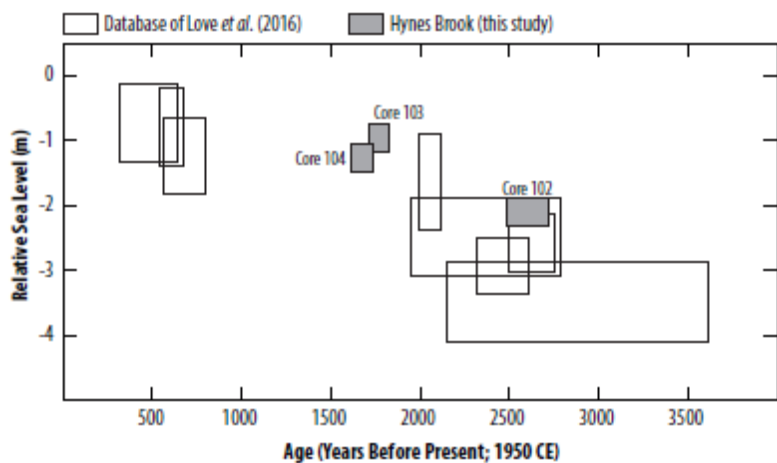

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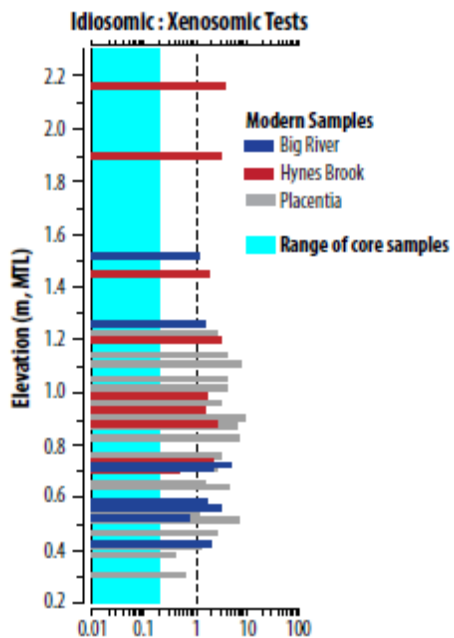1106   Figure 6.

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