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2 **Testate amoeba communities of the drained Hula wetland (Israel): implications**
3 **for ecosystem development and conservation management.**

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1

2 **ABSTRACT**

3

4 This study investigates the testate amoeba communities of semi-aquatic environments
5 in two anthropogenic wetland ecosystems within an extensive drained wetland
6 complex in northern Israel. Aims are to add to the species record for the region, test
7 the similarity in amoeba communities and ecology to more studied sites and regions
8 and investigate processes of wetland development and the implications of this for
9 conservation management. The testate amoeba community is predominantly
10 composed of cosmopolitan taxa but the community composition is distinct from that
11 of previous studies. Redundancy analyses show that much the strongest environmental
12 control is hydrology (depth to water table). Surprisingly, strontium (Sr) is an
13 important secondary control, probably representing the trophic gradient. With a few
14 exceptions the autecology of taxa identified here agrees with their preferences
15 indicated by previous studies. There are significant differences in species richness and
16 community structure between the amoeba communities of the two sites. Partly the
17 difference may be due to differences in nutrient state, although some of the difference
18 is independent of all environmental variables tested here. The lower species richness
19 of the more recently created site suggests the testate amoeba community may be at an
20 earlier successional stage.

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22 **KEYWORDS:** Protists; Peatlands; Wetlands; Restoration; Management; Conservation

23 Running title: Testate amoebae in the Hula wetland

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1

2 INTRODUCTION

3

4 Testate amoebae are a polyphyletic group of microorganisms (protists) which
5 are abundant in wetlands around the world. Testate amoebae are good indicators of a
6 variety of environmental variables including hydrology, pH and nutrient status
7 (Mitchell et al. 2008). As testate amoebae respond rapidly to environmental change,
8 can be readily extracted, and can often be identified to species level, they are
9 increasingly used in biomonitoring, with applications as diverse as investigating the
10 impact of lead pollution (Nguyen-Viet et al. 2008) and chemical weapon disposal
11 (Stoiko et al. 2006). Testate amoebae constitute a large proportion of microbial
12 biomass in wetlands and lie at the top of the microbial foodweb, therefore changes in
13 testate amoeba communities may be indicative of changes throughout the microbial
14 community. Testate amoebae are abundant in both human-impacted as well as natural
15 wetland sites and have been used in studies of peatland restoration, in particular to
16 assess the impacts of peat-cutting using the palaeoecological record to track
17 regeneration processes and set targets for restoration (Buttler et al. 1996; Jauhiainen
18 2002; Davis and Wilkinson 2004). The method has also been used to investigate the
19 affects of peatland management regime, forestry (Hendon and Charman 2004;
20 Vickery and Charman 2004) and inundation (Lamentowicz and Obremaska,
21 submitted). Most recently, Laggoun-Défarge et al. (2008) used testate amoebae to
22 reveal differences among regenerating stages and uncut areas of a cut-over peatland,
23 even where there was little difference in vegetation. In this study we investigate the
24 testate amoeba communities of drained, restored and re-created environments of the

1 Hula Wetland, Israel with the aims of adding to our knowledge of testate amoeba
2 ecology and wetland development processes.

3

4 SITE and METHODS

5

6 The Hula (in Hebrew, also variously transliterated as Hulah, Houla,
7 Huli, Hooleh and Huleh) was a large wetland complex in the Afro-Syrian rift valley of
8 northern Israel (33°04' N, 35°35' E, approximately 70 m asl). Peat deposits started
9 accumulating around 20,000 BP and reached a depth of 8-9 m (Hambright and Zohary
10 1998). The wetland complex consisted of a lake (Lake Hula: c.13 km²) and up to 60
11 km² (seasonally variable) of Papyrus-dominated marshes, mostly to the north of the
12 lake (Fig. 1, Jones 1940; Hambright and Zohary 1998; 1999; Gophen 2004; 2008).

13 In 1951-58 the marshes were drained by digging canals in order to provide
14 economically valuable arable land, reduce the malaria risk and increase water supply
15 by reducing evapotranspiration (Hambright and Zohary 1999). A small area of the
16 lake and marshes (3.5 km²) was enclosed prior to drainage, being designated Israel's
17 first nature reserve in 1964. Following construction of canals the water table of the
18 drained area dropped considerably and was subject to greater seasonal variability, peat
19 was oxidised and removed by wind erosion (Hambright and Zohary 1998; Atzmon
20 and Henkin 1998; Gophen 2004; 2008; 2007). In around 8% of the area agriculture
21 lost its economic viability and the land remained uncultivated, increasing the threat of
22 nutrient fluxes to water quality in Lake Kinneret ('Sea of Galilee'), Israel's largest
23 freshwater lake. About 120 animal species have not been recorded in the Hula since
24 drainage (Dimentman et al. 1992). Faced with these problems a limited rehabilitation
25 programme was instigated from 1993-1997 with the pragmatic aims of reducing

1 nutrient output, limiting soil loss and subsidence and promoting tourism. A new lake
2 (Lake Agmon) was dug within the drained marsh area and a network of barriers and
3 canals constructed to raise the water table of the area.

4 In this study we investigate the testate amoeba communities of three
5 ecosystems of the Hula Valley:

- 6 1. The drained marshes; the extensive area of marsh which was drained in the
7 1950s but has not been re-flooded and continues to be used for arable
8 agriculture.
- 9 2. The Hula Nature Reserve; the small area enclosed by dams during the
10 drainage programme. Although the area has never been intentionally drained it
11 is in a far from natural condition. The dams built to maintain the water level
12 leaked extensively, the water table dropped considerably and the site has been
13 subject to nitrate pollution. New marshes of *Cyperus papyrus* and *Phragmites*
14 *australis* have developed on the bed of the former lake (Dimentman et al.
15 1992). These problems have been addressed through rehabilitation projects
16 since the early 1970s. Although the reserve is currently in an improved state it
17 cannot be considered a true relic of the previous Lake Hula and wetlands.
- 18 3. The area of drained marshes which was the focus of the 1990s restoration
19 programme, the Hula Project and Lake Agmon. A large, shallow, new lake
20 and a network of canals were constructed and marshes spontaneously
21 developed around the water bodies. Since the intervention more than 70 plant
22 species have established themselves in the new wetland and increasing
23 numbers of bird (presently about 300 species: Gophen 2007) and mammal
24 species are using the site. Although viewed as broadly successful the new lake

1 ecosystem is eutrophic and has faced some problems with cyanobacteria
2 blooms (Kaplan et al. 1998).

3

4 Our study is confined to the semi-aquatic habitats of the Hula Nature Reserve marshes
5 and marshes fringing Lake Agmon where testate amoebae are comparatively
6 numerous.

7 The study has three primary aims.

- 8 1. Firstly to simply add species data for the site. A comprehensive attempt to
9 reconstruct the past and present flora and fauna of the Hula has been
10 undertaken (Dimentman et al. 1992) but this does not include any information
11 on testate amoebae.
- 12 2. To investigate the ecology of testate amoebae. This is, to the best of our
13 knowledge, the first study of testate amoebae in a Middle Eastern wetland. We
14 attempt to determine if the community composition and ecological controls on
15 amoeba communities are the same as in more studied ecosystem types (such as
16 ombrotrophic peatlands) and more studied regions (such as northern Europe) ..
- 17 3. To use testate amoebae to investigate the development of the two rehabilitated
18 wetlands. Are the testate amoeba communities of the two sites the same and if
19 not why are they different?

20

21 Initial fieldwork was carried out in September 2007. Forty four samples were
22 extracted from a cross section of the Hula Nature Reserve marshes and 11 samples
23 from around Lake Agmon (Fig. 1). Wetter locations are relatively under-represented
24 in the Lake Agmon data as accessing the areas closest to the water body was
25 extremely difficult. A further seven samples were extracted from an area of the

1 drained marshes, now used for arable agriculture, to the south of Lake Agmon in
2 December 2008 but no tests were found in these samples (Fig. 1).

3 To investigate the environmental controls on amoeba communities,
4 environmental data was collected at each sampling point. In the field, the vegetation
5 of each sampling site was recorded and a sample of surface sediment and leaf litter
6 approximately 2 x 2 x 4cm was removed. Depth to Water Table (DWT) was measured
7 by making a small hole which was left for the water table to equilibrate with the
8 surrounding sediments; such measurements were not obtainable for many of the
9 Agmon samples. In the laboratory a 1 cm³ sediment subsample was suspended in 30
10 ml deionised water and pH and electrical conductivity (EC) measured. Further sub-
11 samples were dried at 110° C and then incinerated at 550° C; weights pre-drying and
12 pre- and post-incineration were used to calculate % moisture and loss on ignition
13 (LOI). Dried sub-samples (approximately 0.2 g) were subjected to microwave-
14 accelerated acid digestion with HNO₃, filtered and diluted. A suite of 22 elements
15 were analysed by ICP-AES, of which 14 (Al, B, Ba, Ca, Cr, Cu, Fe, K, Mg, Mn, Na,
16 Pb, Sr, Zn) were present in measurable concentrations.

17 Testate amoebae were extracted from samples using a slightly modified
18 version of the water-based method of Hendon and Charman (1997). A sub-sample of
19 c.1 cm³ was placed in a beaker with 50 ml of boiling water and stirred to disaggregate.
20 The sample was filtered at 300 µm and then back-filtered at 15 µm with the 15–300
21 µm fraction retained. The sample was left for a day for the particulates to settle out
22 and the supernatant decanted off. The prepared sample was stored in a glass vial and
23 slides prepared by mixing a drop of the material with glycerol on a microscope slide.
24 A count of 150 amoebae per sample was aimed for (minimum=142, mean=151). A

1 conservative taxonomic scheme based on Charman et al. (2000) was adopted; full
2 details are given in Appendix 1.

3 The data structure and links between the species and environmental data were
4 tested by ordination, linear techniques were most appropriate given the short
5 compositional gradients (determined by DCA). Principal Components Analysis
6 (PCA) was used to investigate the general structure of the entire testate amoeba data-
7 set. The ecology of the larger dataset from the Hula Nature Reserve was first
8 investigated on its own. Redundancy Analysis (RDA) was used to determine the
9 major environmental controls on amoeba communities. A total of 20 environmental
10 variables were included in the analysis: DWT, pH, EC, LOI, % moisture, vegetation
11 (expressed as either *Phragmites australis* or *Cyperus papyrus* dominant) and 14
12 geochemical variables. A minimal suite of environmental variables was determined by
13 forward selection with variables exceeding a Bonferroni-corrected P-value included in
14 the model. A sequence of partial RDAs was used to determine the proportion of
15 variance explained by each of these variables independently. All ordination analyses
16 were carried out in CANOCO ver.4.53 (Ter Braak and Šmilauer 1997-2004) and used
17 square-root transformed data. The significance of these results was determined by
18 Monte Carlo Permutation tests (999 permutations).

19 Two approaches were used to test whether there was significant difference in
20 amoeba community between the two sites. An initial test of similarity used Analysis
21 of Similarity (ANOSIM) (Clarke 1993) with a Bray-Curtis distance measure and
22 10,000 permutations in PAST ver.1.71 (Hammer et al. 2001). Subsequently a
23 combined data-set from both sites was analysed by RDA with a site nominal variable
24 to determine the extent of difference between sites and identify the environmental
25 data most closely associated with the site variable. Forward selection was again used

1 to minimise the number of environmental variables, however in these analyses the
2 ‘Site’ variable was pre-selected. To help explore the relationship between amoeba
3 community and hydrology a species-environment (transfer function) model was
4 developed by testing a suite of model structures (Birks 1995) in C² ver. 1.4 (Juggins
5 2003) with outliers removed following Payne et al. (2006).

6

7 RESULTS

8 The data-set

9

10 A total of 27 testate amoeba taxa were identified in the 55 samples with
11 countable test concentrations (Table 1), of which the most abundant were *Trinema*
12 *lineare* (20% of total count), *Diffugia minutissima* type (17%), *Tracheleuglypha*
13 *dentata* (13%), *Phryganella acropodia* type (11%) and *Centropyxis aerophila* type
14 (11%). There is a predominance of small r-strategist taxa that are found in a wide
15 range of locations and environments. The samples were moderately diverse with
16 Shannon diversity ‘H’ ranging from 2.6 to 3.2. Two taxa (*T. lineare* and *T. dentata*)
17 were found in all of the samples. Apparent test concentrations were low; probably due
18 at least as much to the abundance of fine, degraded, organic material as to genuinely
19 low concentrations. There is a high abundance of taxa with very small tests (e.g. *D.*
20 *minutissima* type, *T. lineare*, *Cryptodiffugia oviformis*), as many of these tests have a
21 breadth less than 15µm it is possible that some individuals may have been lost
22 through back-sieving and their abundance under-estimated (Payne 2009). Loss on
23 ignition values averaged 57%, showing that the sediments of the extant wetlands,
24 unlike the pre-drainage marshes, cannot be strictly classified as peat (some samples

1 had values as high as 92% but these high values are likely to be due to the inclusion of
2 overlying leaf litter).

3 Although most of the taxa encountered in this study are found comparatively
4 frequently in testate amoeba studies, the community composition appears distinct
5 from that of previous studies of wetland testate amoebae. Particularly notable is the
6 high abundance of Euglyphidae and total absence of several common genera, most
7 notably *Nebela*. The species composition is clearly distinct from true peatlands
8 including both ombrotrophic peatlands (which have been the most studied ecosystems
9 to date) and also brown moss and sedge dominated minerotrophic peatlands
10 (Opravilova and Hajek 2006; Payne and Mitchell 2007).

11

12 Testate amoeba ecology in the Hula Reserve

13

14 The testate amoeba data were analysed using PCA and RDA. Analyses of the
15 data from the Hula Nature Reserve alone are discussed first, before discussing
16 differences between the Hula Nature Reserve and Lake Agmon data-sets. In the RDA
17 of the Hula Nature Reserve data-set (Fig. 4) alone, only two significant environmental
18 variables (DWT and Sr) were identified in forward selection (collectively explaining
19 46.7% of variance (P=0.001)). When the role of the two variables was separated in
20 variance partitioning, Sr explained 5.9% of variance and DWT explained 37.5%
21 variance (both P=0.001) (Table 3). As hydrology was the most important
22 environmental control on amoeba community structure it was possible to develop a
23 transfer function model to estimate DWT for samples without a measured value. The
24 best performing model structure was found to be Weighted Averaging with inverse
25 deshrinking giving an RMSEP_{jack} of 2.7 cm with two unusual samples removed (Fig.

1 2, Table 2). Although the model performed well in cross-validation of the Hula Nature
2 Reserve samples, there were significant differences between model-predicted and
3 measured DWT values for those Lake Agmon samples with DWT measurements (Fig.
4 2). This suggests there may be some intrinsic differences in amoeba community
5 response to hydrology between the two sites.

6

7 Differences between the two-sites

8

9 Seven taxa were encountered in the Hula Reserve but not in the samples from
10 around Lake Agmon (*Arcella vulgaris* type, *Arcella dentata*, *Diffflugia* ‘type X’,
11 *Diffflugia* cf. *glans*, *Diffflugia* cf. *lacustris*, *Diffflugia oblonga*, *Euglypha compressa* and
12 *Plagiopyxis* spp.) and one taxon (*Trinema complanatum*) in the Lake Agmon samples
13 but not the Hula Reserve. The species data (Table 1) shows major differences in
14 overall abundance between the two sets of samples for several taxa, notably *Arcella*
15 *discoides*, *Centropyxis aculeata* type, *D. minutissima* type, *Diffflugia pulex* type and
16 *Trinema enchelys*. There is no significant difference in diversity between the two sets
17 of samples (permutation t-test [10,000 permutations] on Shannon ‘H’ P=0.76) but
18 there is a significant difference in species richness (permutation t-test P=0.002). The
19 difference in community composition between the two sets of samples emerges very
20 clearly in the PCA (Fig. 3). Agmon samples generally have higher scores on both axis
21 one and axis two. With the exception of a single sample, the Agmon samples form a
22 coherent group on the upper right-hand side of the plot. This difference is confirmed
23 by ANOSIM, showing there is a significant difference between the two multivariate
24 data-sets ($R_{\text{ANOSIM}}=0.28$, $P<0.001$).

1 Using RDA to test difference between the amoeba community of the two sites
2 presents problems due to the differences in the hydrological range represented by the
3 two sets of samples. While there may well be real differences between the
4 hydrological ranges of the two sites, the differences in DWT measurements here
5 largely reflects differences in sampling. As hydrology is the most important control on
6 amoeba communities it is important that differences in DWT be accounted for in
7 comparing the two sets of samples. We test five approaches here: 1) Ignoring DWT
8 values entirely. 2) Excluding all samples without DWT measurements (measured
9 DWT values are termed DWTm). 3) Assigning samples without DWT measurements
10 a ball-park estimate of 25 cm (this dataset is termed 'DWT25'). 4) Using the transfer
11 function derived from the Hula Reserve to estimate DWT in Agmon samples without
12 measured values (termed TI-DWTa). 5) As above but using transfer function
13 estimates for all Agmon samples (termed TI-DWTb). None of these approaches are
14 ideal. Approach 2 leaves a very small sample set (4 samples), Approach 3 is a crude
15 approximation, Approaches 4 and 5 are based on the Hula Reserve transfer function
16 which performs comparatively poorly for Agmon samples with DWT measurements
17 and Approach 1 ignores an important environmental variable. However, by combining
18 a range of approaches it is hoped that it is possible to account for the limitations of
19 each individual method.

20 RDA of the combined data set from both sites shows that water table depth
21 (however assessed) remains the strongest environmental variable but the combination
22 of chemical variables selected in forward selection depends on the approach taken to
23 the lack of DWT measurements from the Agmon site. Analyses variously include Sr,
24 Mg, Fe and Zn. When all data is analysed, the site variable without co-variables
25 explains 16.7% of variance (when only samples with DWT measurements are used

1 this is greatly decreased to 9% (P=0.001 and P=0.011 respectively)). When hydrology
2 is accounted for the proportion of variance explained by the 'Site' variable depends on
3 the approach to dealing with the lack of DWT data for some Agmon samples. If
4 hydrological variables are not considered (Approach 1) 'Site' explains 22.9% of
5 variance, if only measured DWT values are used (Approach 2) 'Site' explains 8% of
6 variance, if an arbitrary value of 25 cm is used (Approach 3) 'Site' explains 5.5 % of
7 variance, if model-predicted values are used for the Agmon samples without DWT
8 measurements (Approach 4) 'Site' explains 7.8 % variance and if model predicted
9 values are used for all Agmon samples (Approach 5) 'Site' explains 7.1 % variance
10 (all P=0.001) (Table 3). These results therefore show that the 'Site' variable explains
11 a significant proportion of variance if DWT is estimated using any of these methods,
12 if only samples with measured DWT values are used and if DWT is ignored entirely.
13 Despite the limitations of each of these approaches it therefore seems highly probable
14 that there is a significant difference between the two sites regardless of hydrology.
15 Fig. 5 shows the RDA plot using Approach 4, DWT estimated by transfer function for
16 samples without measured values, other approaches give similar results.

17

18 DISCUSSION

19 Environmental controls and autecology

20

21 The redundancy analyses show that depth to water table is the most important
22 environmental variable, independently explaining 37.5% of variance in the Hula
23 Reserve data. The importance of hydrology as an environmental control in these sites
24 is unsurprising given its importance for testate amoeba communities in other wetland
25 environments. Wetness (however assessed) has been widely found to be the most

1 significant environmental variable in numerous studies from peatlands (e.g. Woodland
2 et al. 1998; Mitchell et al. 1999; Booth 2002; Payne et al. 2008) and tidal level to be
3 most important in saltmarshes (Charman et al. 2002). Even in the context of this
4 previous research, the independent proportion of variance explained in this study is
5 notably high. Studies in peatlands have found that DWT explained 7% (Charman et
6 al. 2007), 5.8% (Payne et al. 2006), 10% (Payne and Mitchell 2007) and 9.4%
7 variance (Payne et al. 2008).

8 The hydrological preferences of taxa found in this study are in general
9 agreement with results from other wetland environments (Fig. 4). In peatlands
10 *Trinema lineare*, *Cryptodiffugia oviformis* and *Euglypha rotunda* type are most
11 commonly found at the drier end of the hydrological gradient and *A. discoides* and *C.*
12 *aculeata* in wetter positions, consistent with observations here. *P. acropodia* type is
13 negatively correlated with DWT, which is counter to some studies in peatlands;
14 however the group includes many taxa which are likely to have different hydrological
15 preferences so it is difficult to make such comparisons. There is little comparison data
16 for *D. minutissima* type (negatively correlated with DWT), *T. enchelys* or *T. dentata*
17 (both positively correlated with DWT).

18 The importance of Sr as an environmental control on amoeba communities in
19 these datasets is somewhat surprising. Sr independently explains 5.9% of variance in
20 the Hula Reserve dataset and around 4-5% in the overall dataset (depending on
21 approach taken to lack of hydrological data for some Agmon samples). Sr has not
22 been analysed in previous testate amoeba ecology studies but other chemical variables
23 have been shown to be important including Ca, K and Mg (Lamentowicz et al. 2008,
24 Opravilova and Hajek 2006). Sr concentrations in our samples averaged 101 µg/g; by
25 comparison in profiles from three minerotrophic peatlands in Switzerland Sr

1 concentrations ranged between approximately 15 and 250 $\mu\text{g/g}$ (Shotyk et al. 2000)
2 and in surface samples from a *Phragmites* wetland on Cyprus Sr concentrations
3 reached over 1000 $\mu\text{g/g}$ (Payne, unpublished data). Sr concentrations in these
4 sediments therefore appear relatively high but not extremely so. Sr in the Hula
5 wetlands is ultimately derived from the bedrock but Sr concentrations increase
6 markedly downstream from the headwaters to the lower reaches of the Jordan River
7 (Sandler et al. 1988). To investigate the causes of this increase we studied
8 geochemical data collected from the 'Z canal' (directly upstream of Lake Agmon) in
9 2004 (Nishri, unpublished data). These data show a strong correlation between Ca and
10 SO_4 ($R^2=0.95$; Fig. 6) and between Sr and SO_4 ($R^2=0.96$) which we interpret as
11 indicating that both Ca and Sr in the waters of the Hula are derived from leaching of
12 the extensive gypsum deposits in the drained peat soils. The co-precipitation
13 coefficient of strontium with gypsum has been shown to be relatively high (Kushnir
14 1980). The Ca: SO_4 ratio in these water samples is 0.37, close to the ideal ratio of 0.41
15 which would be theoretically expected due to dissolution of gypsum. The correlation
16 with SO_4 is notable given recent suggestions that sulphate may be a relatively
17 important control on testate amoeba communities (Payne et al. in press). In our
18 sediment samples Ca, K and Mg are all highly correlated with Sr, particularly
19 noticeable when considering the Hula Reserve data alone (Fig. 4). It is probable that
20 once Sr is selected in forward selection these other variables lose significance. We
21 suggest that the Sr variable should probably be interpreted as representing the broader
22 trophic gradient. The taxa most closely correlated with Sr are *Centropyxis aerophila*
23 type and *Phryganella acropodia* type (positive correlation), and *Arcella megastoma*,
24 *Diffugia cf. lacustris* and *Arcella vulgaris* type (negative correlation) (Figs. 4 and 5).

1 It is notable that more environmental variables are not identified as significant
2 in forward selection. In peatlands pH has been found to be an important secondary
3 environmental gradient in many studies (Lamentowicz and Mitchell 2005; Payne et al.
4 2006) and even surpasses hydrology in some studies, particularly from minerotrophic
5 sites (e.g. Opravilova and Hajek 2006; Booth et al. 2008). Electrical conductivity
6 (Booth 2007; Booth et al. 2008) and loss on ignition (Payne and Mitchell 2007) have
7 also been shown to be significant environmental variables in some peatland studies.
8 While it may be the case that these environmental variables are less important in sites
9 like these than in peatlands, it is more probable that all these variables are acting as
10 proxies for the base richness gradient, which in this study is represented by Sr.

11

12 Differences between Lake Agmon and Hula Nature Reserve

13

14 There is a clear difference between the amoeba communities of the Hula
15 Nature Reserve and Lake Agmon samples (Table 1, Fig. 5). A variable for site
16 remains significant even when hydrology and chemical variables are accounted for
17 (Table 3). In the RDA plot of the major taxa the 'Site' variable is positively correlated
18 with *A.megastoma*, and negatively correlated with *Cyclopyxis* 'type Z' and *Trinema*
19 *complanatum*, and to a lesser extent *Trinema enchelys* and *Phryganella acropodia*
20 type. Given the limited knowledge of the autecology of these taxa it is difficult to
21 provide any ecologically meaningful explanation for why their abundances are so
22 different in these two sites. It is interesting to note that *A. discoides* is significantly
23 more abundant in the samples from around Lake Agmon than in the Hula Reserve.
24 Recent research in Polish peatlands (Lamentowicz et al. 2008, in press) has suggested
25 that this taxon may be an indicator of disturbance and fluctuating water tables.

1 The 'Site' variable is strongly negatively correlated with various chemical
2 variables which were not selected in forward selection (Ca, EC). The concentration of
3 Ca is considerably higher in the Lake Agmon samples, despite the presence of
4 calcareous basal sediments in the Hula Nature Reserve. This is presumably because the
5 lateral and vertical development of the marsh sediments has reduced the contact
6 between the surficial sediments and the hard water supplied by the River Jordan. As
7 can be seen in the RDA plot (Fig. 5) there is some co-variance between the 'Site' and
8 'Sr' variables, this explains 1% of overall variance in this example. Lake Agmon
9 samples were more nutrient rich but there is still significant difference between the
10 sites even when these differences are accounted for.

11 The difference between the Hula and Agmon data might be explained by some
12 other environmental variables which were not determined in this study. Although this
13 study includes a large number of environmental variables it is possible that other
14 variables such as Bulk density, NO₃, PO₄ and SO₄ could also be significant controls
15 on amoeba communities, analysis of these further variables was precluded by sample
16 size. However, the study of Lamentowicz et al. (2008), which did include many of
17 these additional variables, found that only pH, Mg and DWT were selected in forward
18 selection so additional variables would not necessarily have explained additional
19 variance here.

20 One apparent difference between the two amoeba communities is the species
21 richness. The Hula Reserve samples have a greater species richness than the Agmon
22 samples (mean=9.8 against mean=11.6) and this difference is highly significant.
23 While it is possible that this difference might relate to environmental differences
24 between the sites, no previous studies have (to our knowledge) suggested that species
25 richness varies along either the trophic or hydrological gradients. An alternative

1 possible cause is the difference in the age of the two ecosystems. While the Agmon
2 ecosystem was only created by pool excavation in the 1990s the Hula Reserve has,
3 broadly speaking, been in its current form since the 1970s and has a continuous
4 history back to before the drainage of the original marshes and lake. Studies of testate
5 amoeba primary succession show a gradual increase in species richness over time
6 with little or no replacement (Lousier 1982; Wanner and Xylander 2005). The
7 difference in species richness suggests that the amoeba community of the Agmon
8 Reserve might still contain vacant niches which have not yet been colonised despite
9 the presence of amoeba communities adapted to wetland environments in the
10 surrounding area. It is notable that many of the taxa found in the Hula Reserve but not
11 the Agmon site have intermediate- to large-sized tests (none have tests lengths below
12 c.70 μm), and may therefore be expected to have more restricted distributions (cf.
13 Wilkinson 2001). By contrast, the only taxon found in the Agmon Reserve but not the
14 Hula Reserve (*T. complanatum* type) is a small generalist that is often found in drier
15 niches which may not have been sampled in the Hula Reserve.

16

17 CONCLUSIONS

18

19 In terms of floristic composition and appearance the new marshes fringing
20 Lake Agmon are essentially identical to many areas of the more established Hula
21 Nature Reserve (and to areas of the original Hula marshes); a tall, dense monoculture
22 of *Phragmites australis*. Analysis of testate amoebae shows that despite this apparent
23 similarity, the environment as experienced by microorganisms is quite different. The
24 Agmon marshes are more nutrient-rich and the testate amoeba community appears to
25 be at an earlier successional stage. This shows the value of including micro-organisms

1 in studies of wetland restoration. Whether or not the testate amoeba communities of
2 the two environments will eventually converge is an open question. It would be
3 interesting to also test the similarity in amoeba community between these two sites
4 and the original Hula marshes. However, we have been unable to locate any sediment
5 samples preserved from before drainage, and even if any such samples were available
6 the uncertain preservation of tests might well compromise a comparison (cf. Payne
7 2007).

8 Testate amoeba communities of these sites are strongly controlled by
9 hydrology. The transfer function model developed here may allow palaeoecological
10 reconstruction of post-drainage hydrological change in the Hula Nature Reserve. It is
11 possible that the model could also be used to reconstruct the longer-term history of
12 hydrological change in the Hula, although the preservation of tests is likely to be poor
13 and this may be impossible in practise.

14 The importance of Sr in this study is an interesting finding. Sr concentrations
15 probably represent the trophic gradient, and while it is not surprising that this gradient
16 is an important control on amoeba communities it is surprising that this is better
17 represented by Sr than by other elements such as Ca or Mg. It will be worth analysing
18 Sr in future studies of testate amoeba ecology.

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9 1 was prepared by Graham Bowden. Thanks to two anonymous reviewers for

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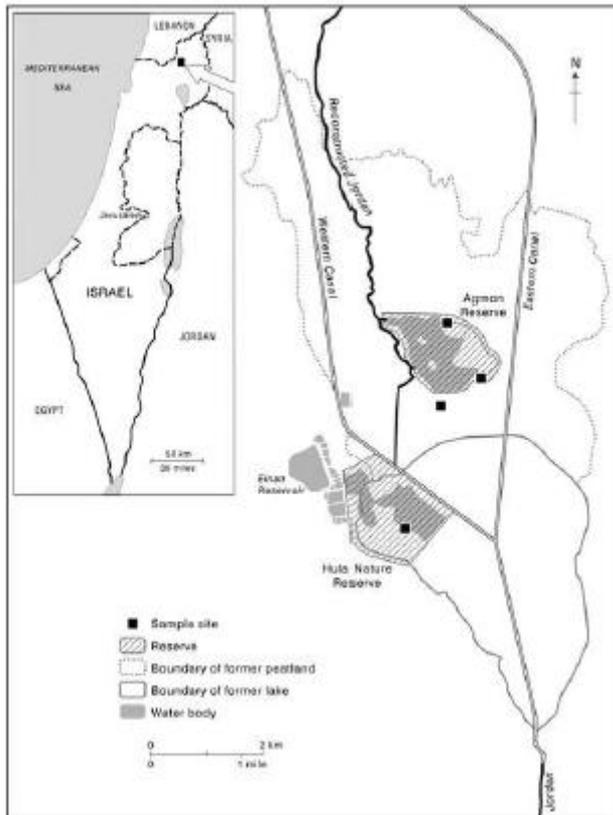
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1 FIGURES

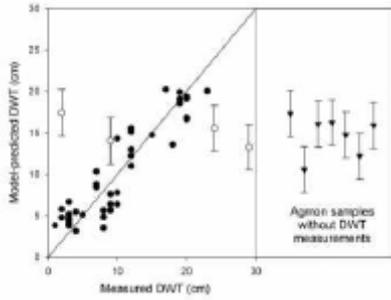
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3 Fig. 1. Map showing location of Hula valley within Israel, location of Lake Agmon
4 and Hula Nature Reserve, outlines of former lake and marshes and position of
5 sampling areas within the sites.



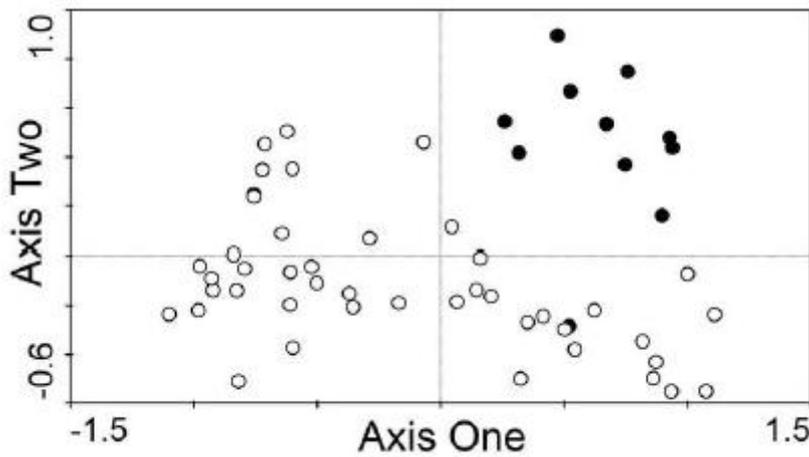
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7 Fig. 2. Transfer function model performance showing: model predicted against
8 measured DWT values for Hula Reserve samples (black circles); model predicted
9 against measured DWT values with boot-strapped (1000 cycles) standard error
10 estimates for Agmon samples (white circles) and model predicted DWT values and
11 errors for Agmon samples without measured DWT values (black triangles, inset).
12 Solid line is 1:1, the ideal relationship between model predictions and measured
13 values.



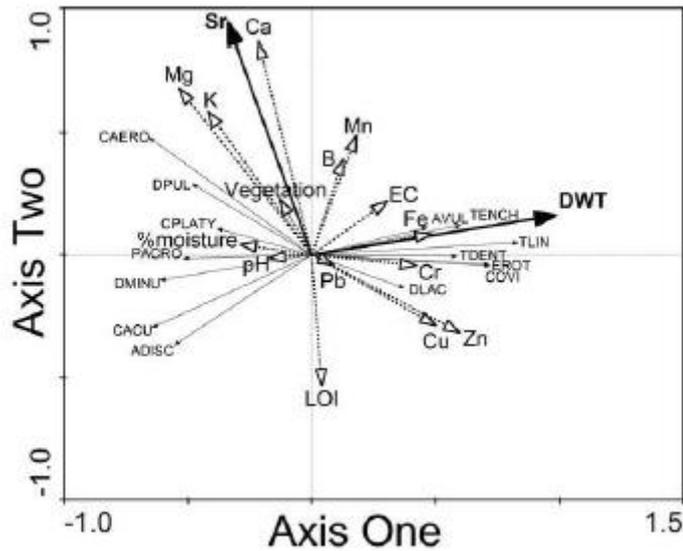
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2 Fig. 3. Principal components analysis (PCA) of testate amoeba data. Samples marked
 3 in white are from the Hula Nature Reserve, samples marked in black are from Lake
 4 Agmon.



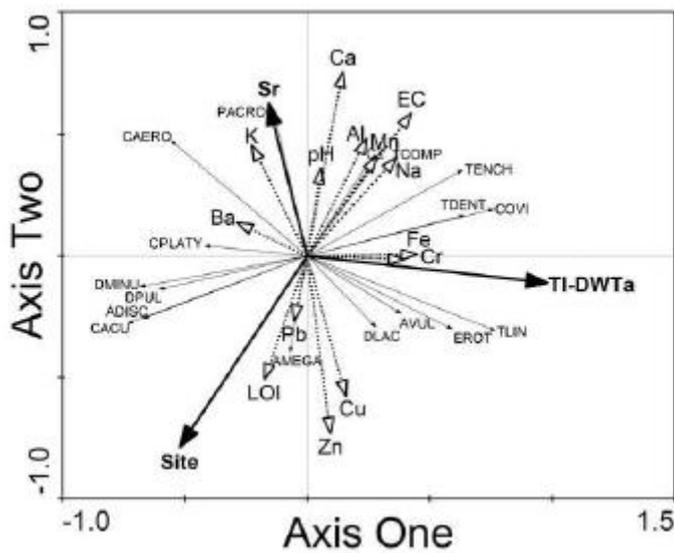
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6 Fig. 4. RDA plot based on square-root transformed data from Hula Nature Reserve.
 7 Showing major species (narrow solid lines), significant environmental variables (thick
 8 solid lines) and other environmental variables passively projected (dotted lines).
 9 Species codes are given in Table 1. Environmental variable codes: depth to water
 10 table (DWT), loss on ignition (LOI), *Phragmites* or Papyrus dominant (Vegetation),
 11 electrical conductivity (EC), percent moisture (% moisture) and chemical variables
 12 shown by standard abbreviations.



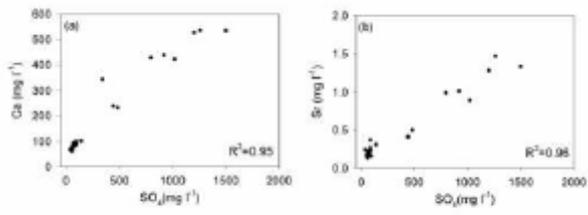
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2 Fig. 5. RDA plot for all data, details as for Fig. 4.



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4 Fig. 6. Geochemical data for water samples from the 'Z canal' collected between
 5 January and November 2004. (a) Ca against SO_4 , and (b) Sr against SO_4 . We interpret
 6 these correlations as suggesting both Ca and Sr in these samples are derived from
 7 dissolution of gypsum.



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TABLES

Table 1. Abundances of major taxa (>1% overall total) in Hula Nature Reserve and Lake Agmon. Also showing species codes used in Figs. 4 and 5.

Taxon ¹	Code	% in Hula Reserve	% in Agmon Reserve
<i>Arcella discoides</i> Ehrenberg 1872 type	ADISC	0.3	3.9
<i>Centropyxis aculeata</i> Ehrenberg 1830 type	CACU	0.4	9.6
<i>Centropyxis aerophila</i> Deflandre 1929 type	CAERO	8.5	11.3
<i>Centropyxis platystoma</i> Penard 1890 type	CPLATY	0.8	1.8
<i>Cryptodifflugia oviformis</i> Penard 1890	COVI	7.2	1.6
<i>Difflugia minutissima</i> Penard 1904 type	DMINU	2.8	19.5
<i>Difflugia pulex</i> Penard 1902 type	DPUL	0.1	3.8
<i>Euglypha rotunda</i> Wailes & Penard 1911 type	EROT	4.9	6.3
<i>Phryganella acropodia</i> Hertwig & Lesser 1874 type	PACRO	18.5	8.8
<i>Tracheleuglypha dentata</i> Vejdovsky 1882	TDENT	25.4	9.1
<i>Trinema enchelys</i> Penard 1878	TENCH	6.7	1.0
<i>Trinema lineare</i> Penard 1890	TLIN	20.3	19.9

8 ¹ Minor taxa not shown are: *Arcella vulgaris* Ehrenberg 1830 (AVUL), *Arcella dentata* Ehrenberg 1830 (ADENT),
9 *Arcella hemispherica* Perty 1852 (AHEM), *Arcella megastoma* Penard 1902 (AMEGA), *Cyclopyxis* 'type Z' (CZ),
10 *Difflugia* 'type X' (DX), *Difflugia* cf. *glans* Penard 1902 (DGLANS), *Difflugia* cf. *lacustris* Penard 1899 (DLAC),
11 *Difflugia oblonga* Ehrenberg 1832 (DOBL), *Euglypha compressa* Carter 1864 (ECOMP), *Euglypha tuberculata*
12 Dujardin 1841 (ETUB), *Plagiopyxis* spp (PLAG), *Paraquadrula* undiff. (PARA), *Trigonopyxis arcula* Leidy 1879
13 (TARC), *Trinema complanatum* Penard 1890 type (TCOMP). See appendix for notes on taxonomy.

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2 Table 2. Performance of transfer function model showing root mean squared error of
3 prediction (RMSEP), maximum bias (Max Bias), and R^2 assessed by boot-strapping
4 ('boot') and jack-knifing ('jack').

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Model structure	Samples	Taxa	RMSEP_{jack} (cm)	RMSEP_{boot} (cm)	Max Bias_{jack} (cm)	Max Bias_{boot} (cm)	R^2_{jack}	R^2_{boot}
Weighted Average (inverse deshrinking)	42	26	2.7	2.8	3.6	3.7	0.80	0.81

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2 Table 3. Results of redundancy analysis

DWT approach for Agmon Samples*	Data set	No. samples	Explanatory variables	Co-variables	% variance explained	P
-	Hula Reserve samples only	55	DWT, Sr	-	46.7	0.001
-	All samples	55	Site	-	16.7	0.001
-	All samples with DWT measurements	48	Site	-	9	0.011
1	All samples	55	Site	Zn	22.9	0.001
2	All samples with DWT measurements	48	Site	DWTm, Mg, Sr	8.1	0.001
3	All samples	55	Site	DWT25, Fe, Sr	5.5	0.001
4	All samples	55	Site	TIDWTa, Sr	7.8	0.001
5	All samples	55	Site	TI-DWTb, Fe, Sr	7.1	0.001

3 * Methodology used to account for lack of measured DWT values in analysis. See text for details.

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2 APPENDIX 1

3 Details of unidentified taxa and taxonomic groupings used in this study.

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Name	Synonymy
<i>Arcella vulgaris</i> type	Follows Charman et al. (2000).
<i>Arcella discoides</i> type	Follows Charman et al. (2000).
<i>Centropyxis aculeata</i> type	Follows Charman et al. (2000).
<i>Centropyxis aerophila</i> type	Includes all sub-rounded centropyxidae with ovoid sub-terminal aperture. Synonymous with <i>Centropyxis cassis</i> type of Charman et al. (2000).
<i>Centropyxis platystoma</i> type	Follows Charman et al. (2000).
<i>Cyclopyxis</i> 'type Z'	An unidentified species of <i>Cyclopyxis</i> (?); test a shallow disc composed of fine particles c.120 µm diameter with a round approximately central aperture approx 20 µm diameter.
<i>Diffflugia minutissima</i> type	Very small ovoid <i>Diffflugia</i> tests (<20µm length). There may be some overlap between this type and <i>Diffflugia pulex</i> type
<i>Diffflugia</i> 'type X'	A <i>Diffflugia</i> test approx 60µm length with a rounded posterior and round cross-section. Distinguished by a curious 'flared' aperture. The few individuals observed were in a poor state of preservation.
<i>Diffflugia</i> cf. <i>glans</i>	A test which generally agrees with the description of this species in Ogden (1983), although the aperture is markedly wider (30+ µm).
<i>Diffflugia</i> cf. <i>lacustris</i>	Generally in good agreement with descriptions, all tests parallel-sided not pyriform.
<i>Diffflugia pulex</i> type	Small ovoid <i>Diffflugia</i> tests (approx 20-40µm length).

<i>Euglypha rotunda</i> type	Follows Charman et al. (2000). Includes <i>E.laevis</i> .
<i>Phryganella acropodia</i> type	Synonymous with the <i>Cyclopyxis arcelloides</i> type of Charman et al. (2000). Includes all 'bowl-shaped' tests.
<i>Plagiopyxis</i> spp.	Includes all <i>Plagiopyxis</i> species.
<i>Paraquadrula</i> undiff.	All <i>Paraquadrula</i> species.
<i>Trigonopyxis arcula</i> type	In general agreement with descriptions of this species but none of the individuals encountered had a triangular aperture; apertures were irregular but approximately round.
<i>Trinema complanatum</i> type	Includes <i>Trinema penardi</i> .

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