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ROTIFERA XIV

Taxonomic distinctness indices for discriminating patterns in freshwater rotifer assemblages

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Abstract Taxonomic distinctness indices measure the taxonomic relatedness among species and have been used for environmental assessment to detect disturbed habitats. This is the first application of the Average Taxonomic Distinctness (Δ^+) and Variance in Taxonomic Distinctness (Λ^+) indices to the presence/absence data of rotifer communities to examine their sensitiveness in discriminating perturbed environments. The 26 Greek lakes studied spanned a wide range of morphological and physical–chemical characteristics. Δ^+ was significantly correlated ($P < 0.05$) with maximum depth, salinity and trophic state, while Λ^+ was correlated only with salinity. The index Δ^+ identified lakes characterized by periods of increased salinity. Communities in these lakes were less diverse, consisting of more closely related species as seen by the reduced number of families than other lakes with similar species richness. Lakes identified by Λ^+ had a higher community distinctness than expected due to the overrepresentation of the family Brachionidae; they were also characterized by periods of water-level fluctuations. Both indices were unaffected by sampling effort in terms of number of species and sampling visits; whereas Shannon diversity index (H') was correlated to species number. Also, based on the randomization test, the taxonomic distinctness indices differentiated lakes anthropogenically disturbed based on the expected patterns of diversity of the area.

Keywords Rotifera · Taxonomic distinctness · Diversity · Greek lakes · Mediterranean

Introduction

The phylum Rotifera comprised microscopic invertebrates that play an important role in the aquatic food web. Due to their short generation time and their reproductive mode, rotifers show rapid local adaptations (Wallace et al., 2006) making them useful indicators of environmental change. Furthermore, rotifers have been used in aquatic ecotoxicology (e.g. Snell & Joaquim-Justo, 2007), in assessing the trophic state of lakes (e.g. Ejsmont-Karabin, 2012) and in providing information about water quality (Azémar et al., 2010), but they have been neglected in recently developed multimetric indices for the assessment of ecological water quality (Moss et al., 2003; Kane et al., 2009). Based on the taxonomic relatedness among

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species, Warwick & Clarke (1995) introduced an alternative measure of biodiversity; this was taxonomic distinctness, which measures the average degree to which individuals of an assemblage are related to each other. Based on the above concept, Warwick & Clarke (1998, 2001) described two indices, Average Taxonomic Distinctness (Δ^+) and Variation in Taxonomic Distinctness (Λ^+) that can be applied on the presence/absence of the data. Moreover, these indices are independent of sampling effort and sample size (Leonard et al., 2006; Costa et al., 2010). As such, these indices are suitable for comparing species lists from different studies with different sampling effort, even for historic data (Schweiger et al., 2008). A further advantage of these indices is the randomization test that allows comparisons between an observed taxonomic distinctness measure and its expected range of variation (Clarke & Warwick, 1998; 2001). The randomization test is based on the expectation that a random selection from a regional species pool (hereafter called the master species list) determines a baseline against which biodiversity patterns change (Warwick & Somerfield, 2015). Thus, variability in biodiversity due to natural environmental factors generally falls within a predictable range, while anthropogenic perturbation modifies the expected pattern reducing biodiversity (Leonard et al., 2006). Based on this, it has been proposed that taxonomic distinctness indices can be appropriate indicators of the effects that anthropogenic disturbances have on biodiversity over a range of spatial and temporal scales (Leonard et al., 2006).

Taxonomic distinctness indices have shown sensitiveness in discriminating perturbed environments in marine systems (e.g. Clarke & Warwick 1998; Leonard et al., 2006), mainly, but also in estuaries (e.g. Tweedley et al., 2015) and subterranean areas (Gallão & Bichuette, 2015). When applied to freshwater systems, they have not always been correlated with anthropogenic degradation. Bhat & Magurran (2006), using fish community data, could not identify polluted sites in rivers whereas, in contrast, Leira et al. (2009), using data of diatom assemblages, was able to correlate them with eutrophication due to increased nutrient availability in lakes. It has been argued that the effectiveness of these indices is influenced by the special features of the different communities such as endemicity, dispersal ability, tolerance range to different parameters and even the taxonomic system (e.g. Ellingsen et al., 2005; Abellán et al., 2006; Heino et al., 2007).

Rotifers comprise more than 2,000 species and can occur in high densities. Due to their variable adaption and colonization ability, they can be found in both marine and freshwater systems, from large permanent lakes to small temporal puddles, from natron to acidic lakes, and from hyperoligotrophic lakes to sewage ponds (Fontaneto et al., 2006; Segers, 2008). Because of their taxonomic variety and capacity for adaption to different habitats, we examined the sensitiveness of the taxonomic distinctness indices (Δ^+, Λ^+) of rotifer communities in discriminating disturbed environments. We tested the hypothesis that the species present at any lake represent a random selection from the master species list except in perturbed environments. We evaluated the departure of rotifer distinctness of the studied lakes from the expected values using the randomization test and studied taxonomic indices variation in relation to the morphological and physical–chemical characteristics of the lakes. In addition, we estimated commonly used indices, namely the Shannon diversity index (H') and Pielou's evenness (J'), to demonstrate the advantages of the taxonomic distinctness indices.

Materials and methods

Data collection

In our analysis, we used data from 26 Greek lakes (Fig. 1). The lakes encompass a wide range of surface area, maximum depth, salinity as indicated by conductivity and trophic state as indicated by mean summer phytoplankton biomass (Table 1); all of the lakes are alkaline (pH >8). The dataset we used comprised published data from 1987 to 2011 and current data from 2012 to date. The number of samples ranged from 1 to 32 (Online Resource 1—Supplementary materials). The sampling protocol followed before 2012 is well described by Mazaris et al. (2010) and Moustaka-Gouni et al. (2014). Samples from 2012 onwards were collected from the water column in the deepest part of the lake using plankton nets of 50 μm mesh size and preserved in 4% formalin (final concentration). At least 400 individuals per sample were counted for abundance estimation. Rotifers, except Bdelloidea, were identified to the lowest

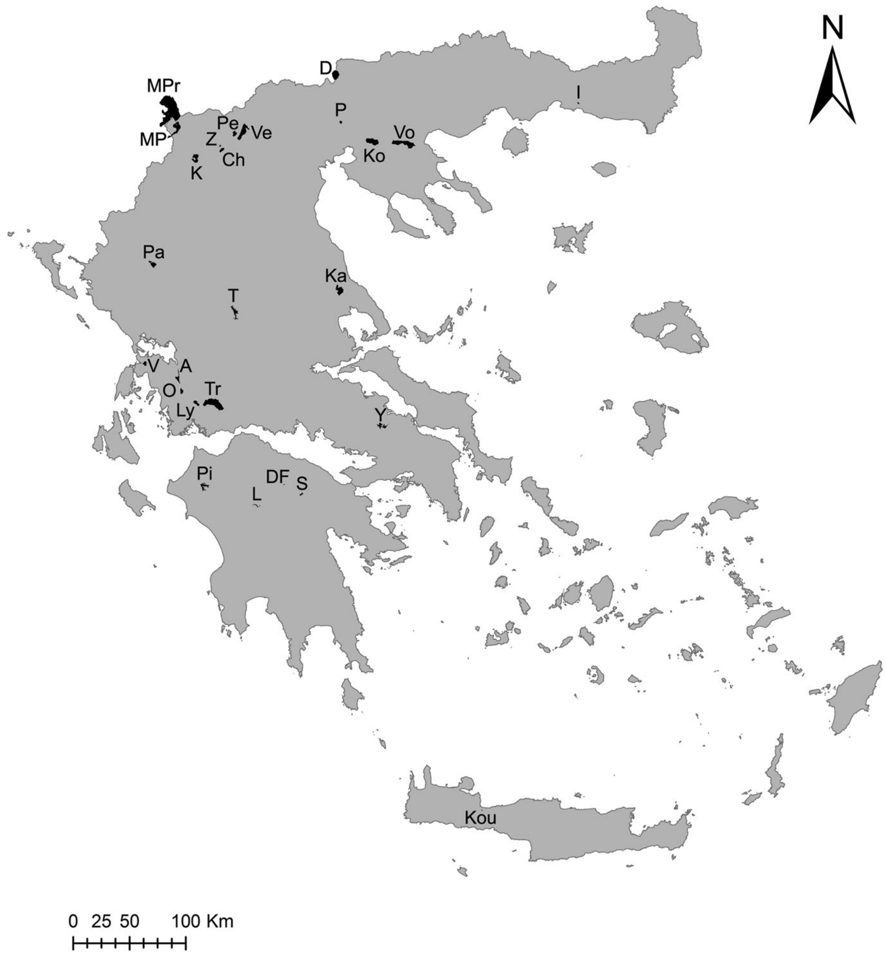

Fig. 1 Map of Greece showing the locations of the 26 lakes included in the study. *Abbreviations* Amvrakia (A), Cheimaditida (Ch), Doirani (D), Doxa-Feneou (DF), Ismarida (I), Karla (Ka), Kastorias (K), Koronia (Ko), Kournas (Kou), Ladona (L), Lysimachia (Ly), Megali Prespa (MPr), Mikri Prespa (MP), Ozeros (O), Pamvotida (Pa), Petron (Pe), Pikrolimni (P), Piniou (Pi), Stymfalia (S), Tavropos (T), Trichonida (Tr), Vegoritida (Ve), Volvi (Vo), Voulkaria (V), Yliki (Y), Zazari (Z)

possible taxonomic level (genus or species) using the taxonomic keys of Koste (1978), Nogrady et al. (1995), Segers (1995) and Nogrady & Segers (2002).

Diversity indices

We applied two taxonomic distinctness indices. The first was the Average Taxonomic Distinctness Δ^+ index, which is the average path length through a taxonomic tree, based on Linnean classification taxonomy, connecting every pair of species in the tree (Clarke & Warwick, 1998). The second was the index of Variation in Taxonomic Distinctness Λ^+, which reflects the unevenness of the taxonomic tree and is the variance of the path lengths between every pair of species in the list (Clarke & Warwick, 2001). The above indices were calculated based on presence/absence data according to the following equations:

$$\Delta^+ = \frac{\left[\sum\sum_{i<j}\omega_{ij}\right]}{\left[\frac{s(s-1)}{2}\right]} \quad \text{and}$$

$$\Lambda^+ = \frac{\left[\sum\sum_{i<j}\left(\omega_{ij}-\Delta^+\right)^2\right]}{\left[\frac{s(s-1)}{2}\right]},$$

where ω_{ij} is the distinctness weight given to the path length linking species *i* and *j* in the taxonomic tree, and s is the number of species present in the sample (Clarke & Warwick, 1998, 2001).

The taxonomic distinctness indices were applied to presence/absence rotifer community data from 26 Greek lakes. Each lake was considered as a sample with the species list consisting of the species present during the study period. The taxonomic tree consisted of six taxonomic levels, namely species, genus, family, order, superorder and class, and was based

Table 1 Morphological and physical–chemical characteristics of the 26 Greek lakes used in the study

Lakes	Maximum depth (m)	Surface area (km^2)	Conductivity[a] (mS cm^{-1})	Phytoplankton biomass[b] (mg l^{-1})	Literature[c]
Amvrakia	37.0	14.2	0.98	–	
Cheimaditida	2.5	10.8	0.39	33.99	2
Doirani	5.5	28.0	0.88	22.61	3
Doxa-Feneou	40.0	5.0	0.35	0.02	1
Ismarida	1.5	2.5	40.05	54.19	4
Karla	2.9	37.4	4.88	20.75	1
Kastorias	8.0	24.0	0.34	12.08	5
Koronia	1.0	42.5	18.10	21.00	6
Kournas	22.5	0.4	1.88	0.76	1
Ladona	50.0	1.5	0.41	0.87	1
Lysimachia	9.0	13.5	0.37	13.97	1
Megali Prespa	50.0	266.0	0.35	5.80	7
Mikri Prespa	8.4	48.5	0.30	29.76	8
Ozeros	2.0	10.1	0.32	2.31	1
Pamvotida	11.0	19.4	0.34	–	
Petron	3.5	8.0	0.95	9.16	9
Pikrolimni	1.0	3.8	83.30	–	
Piniou	43.0	19.5	0.37	0.52	1
Stymfalia	5.0	3.5	0.31	–	
Tavropos	70.0	21.6	0.23	0.16	8
Trichonida	58.0	96.5	0.36	–	
Vegoritida	40.0	39.7	0.69	1.85	8
Volvi	19.0	68.6	1.09	2.80	8
Voulkaria	2.5	9.2	1.27	21.40	1
Yliki	20.0	19.6	0.37	40.27	8
Zazari	3.0	1.9	0.18	12.01	2

[a] Measurements were carried out by the Greek Biotope/Wetland Centre in the framework of the National Monitoring Network of Water Quality and Quantity (defined by the Common Ministerial Decree 140384/2011) under the coordination and supervision of the Special Secretariat for Water. Funded by the European Regional Development Fund and National Resources—Operational Programme "Environment and Sustainable Development 2007–2013"

[b] Phytoplankton biomass refers to mean summer biomass values, (–) indicates that measures of phytoplankton biomass are not available for the period that zooplankton was sampled (Table S1—Supplementary materials)

[c] References for phytoplankton biomass: 1 Moustaka-Gouni & Katsiapi (unpublished), 2 ROP of Western Macedonia (2001), 3 Polykarpou (2006), 4 Moustaka-Gouni et al. (2011), 5 Moustaka-Gouni et al. (2006), 6 Michaloudi et al. (2012), 7 Katsiapi et al. (2012), 8 Moustaka-Gouni et al. (2014), 9 Moustaka-Gouni (unpublished)

on equal step lengths. So, species connected at the highest level had a value of $\omega = 100$. The Rotifer World Catalog (Jersabek & Leitner, 2013) was used to confirm all taxonomic information (i.e. spellings, synonyms, valid names). The master species list was compiled from records of all rotifer species that have been recorded from freshwater systems in Greece (Michaloudi et al., unpublished), studies included in Online Resource 1—Supplementary materials and Zarfdjian & Economidis (1989). The randomization test was applied to both indices to test the null hypothesis that the species present in any lake represent a random selection from the master species list (Clarke & Warwick, 1998, 2001). The indices were quantified and the randomization test was conducted using the TAXDTEST procedure in the PRIMER-E

(Plymouth Routines In Multivariate Ecological Research) v.6 software package (Clarke & Gorley, 2006).

Acknowledging that cases of Genus sp. existed in older studies we also performed the analyses using two different master species lists to account for the effect of different decisions on handling these cases (Online Resources 2 and 3—Supplementary materials): (1) the genera *Collotheca*, *Conochilus* and *Synchaeta*, were considered as a single Genus sp., i.e. *Collotheca* sp., *Conochilus* sp. and *Synchaeta* sp. because they were not identified to species level in the majority of the 26 lakes; (2) all Genus sp. cases were excluded from the master species list. In addition, we performed the analyses based on a taxonomic tree consisting of five taxonomic levels (genus to class) without changing the master species list.

Commonly used diversity indices, namely Shannon diversity index (H') and Pielou's evenness (J'), were calculated based on abundance data if these data were available. For each lake the dataset consisted of the sum of abundances for each species recorded during all sampling visits. The values of H' and J' were quantified using the DIVERSE procedure in the PRIMER-E v.6 software package (Clarke & Gorley, 2006).

Statistical analysis

Linear regression was applied to test the independence of the diversity indices (Δ^+, Λ^+, H' and J') from sampling effort. Sampling effort was estimated with the use of two proxies, the number of species and the number of sampling visits.

In order to determine whether indices Δ^+ and Λ^+ differ significantly with maximum conductivity (salinity) and trophic state, the lakes were classified into two categories according to their maximum conductivity (0–2 and 4–90 mS cm^{-1}) and four categories according to their trophic state (oligotrophic 0.02–1 mg l^{-1}, mesotrophic 1–6 mg l^{-1}, eutrophic 7–15 mg l^{-1}, hypereutrophic 20–55 mg l^{-1}) as determined from their phytoplankton biomass (Table 1). The mean summer values for phytoplankton biomass during the same period as zooplankton samples were collected and were used as a proxy of trophic state because nutrient data were not available. Values for maximum conductivity were the maximum value recorded in each lake up to the study period to indicate freshwater lakes with periods of increased salinity. Kruskal–Wallis test and Bonferroni test were applied to reveal if the taxonomic distinctness indices (Δ^+ and Λ^+) and the traditional indices (H' and J') differed between groups based on the above parameters. Weight cases for each parameter were used to reduce bias due to there being different number of lakes in each group. Linear regression was applied in order to determine whether the taxonomic distinctness indices (Δ^+ and Λ^+) and the traditional indices (H' and J') were significantly correlated with maximum depth and surface area. All statistical analyses were performed using IBM SPSS Statistics 22.

Results

The master species list consisted of 142 rotifer species. These have been classified into 39 genera, 20 families, four orders, two superorders and one class. The dataset consisting of the rotifer communities from the 26 Greek lakes were used to calculate the taxonomic distinctness indices. These included 111 species belonging to 34 genera, 20 families, four orders, two superorders and one class (Online Resource 2—Supplementary materials).

Average Taxonomic Distinctness Δ^+ varied, ranging from 39.35 for Lake Karla to 72.22 for Lake Doxa-Feneou (Table 2). Variation in Taxonomic Distinctness Λ^+ ranged from 153.34 for Lake Stymfalia to 734.48 for Lake Koronia (Table 2). Shannon diversity index (H') varied from 0.94 for Lake Tavropos to 3.23 for Lake Cheimaditida (Table 2). Pielou's evenness (J) ranged from 0.34 for Lake Yliki to 0.80 for Lake Piniou (Table 2). All indices were not correlated significantly with the number of sampling visits (Δ^+: $R^2 = 0.07$, $P = 0.19$, Λ^+: $R^2 = 0.01$, $P = 0.65$, H': $R^2 = 0.03$, $P = 0.43$ and J': $R^2 = 0.03$, $P = 0.41$), whereas only H' was correlated significantly with the number of species (Δ^+: $R^2 = 0.03$, $P = 0.39$, Λ^+: $R^2 = 0.05$, $P = 0.29$, H': $R^2 = 0.56$, $P < 0.001$, J': $R^2 = 0.0003$, $P = 0.94$) (Fig. 2).

Δ^+ differentiated significantly between the categories of conductivity ($H = 17.05$, $P < 0.0001$) and trophic state ($H = 26.87$, $P < 0.0001$) and it was significantly correlated with maximum depth ($P < 0.05$) (Fig. 3). Λ^+ differentiated significantly between the categories of conductivity ($H = 7.16$, $P < 0.05$) (Fig. 4). H' was not significantly correlated

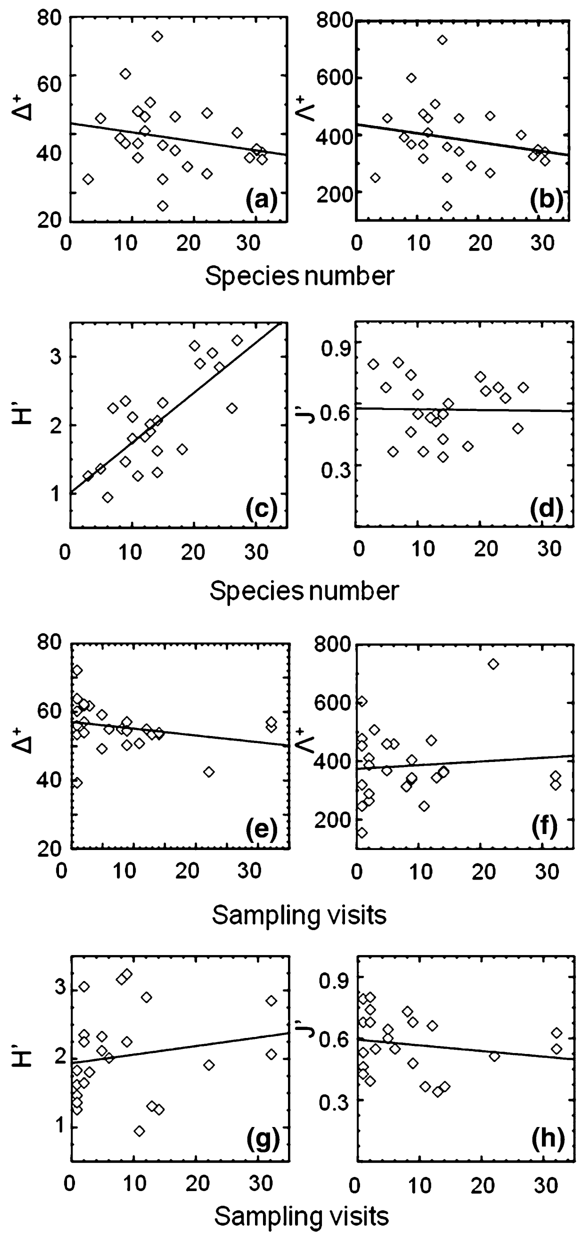

Fig. 2 *Scatter plot* of (**a**) Average Taxonomic Distinctness ▶ (Δ^+), (**b**) Variation in Taxonomic Distinctness (Λ^+) for 26 Greek lakes, (**c**) Shannon index (H') and (**d**) Pielou's evenness (J') for 23 Greek lakes against the number of species (S), and (**e**) Average Taxonomic Distinctness (Δ^+), (**f**) Variation in Taxonomic Distinctness (Λ^+) for 26 Greek lakes, (**g**) Shannon index (H') and (**h**) Pielou's evenness (J') for 23 Greek lakes against the number of sampling visits (SV). The relationships were fitted with linear regressions (**a**) $\Delta^+ = 57.7658 - 0.1368$ S ($R^2 = 0.03$, $P = 0.39$), (**b**) $\Lambda^+ = 436.967 - 3.0813$ S ($R^2 = 0.05$, $P = 0.29$), (**c**) $H' = 1.0143 + 0.0731$ S ($R^2 = 0.56$, $P < 0.001$), (**d**) $J' = 0.57595 - 0.0003$ S ($R^2 = 0.0003$, $P = 0.94$), (**e**) $\Delta^+ = 57.1155 - 0.1973$ SV ($R^2 = 0.07$, $P = 0.19$), (**f**) $\Lambda^+ = 374.4287 + 1.2679$ SV ($R^2 = 0.01$, $P = 0.65$), (**g**) $H' = 1.9389 + 0.0125$ SV ($R^2 = 0.03$, $P = 0.43$), (**h**) $J' = 0.5941 - 0.0027$ SV ($R^2 = 0.03$, $P = 0.41$)

Table 2 Number of species (S), Average Taxonomic Distinctness (Δ^+), Variation in Taxonomic Distinctness (Λ^+), Shannon diversity index (H') and Pielou's evenness (J') for rotifer communities from the 26 Greek lakes used in the study

Lakes	S	Δ^+	Λ^+	H'	J'
Amvrakia	17	58.95	461.23	2.33	0.60
Cheimaditida	30	54.52	344.82	3.23	0.68
Doirani	22	55.05	469.92	2.90	0.66
Doxa-Feneou	3	72.22	246.91	1.25	0.79
Ismarida	27	50.43	404.22	2.25	0.48
Karla	9	39.35	604.21	1.47	0.46
Kastorias	31	54.98	312.69	3.17	0.73
Koronia	14	42.31	734.48	1.91	0.52
Kournas	5	60.00	455.56	1.36	0.68
Ladona	22	57.00	267.28	3.06	0.68
Lysimachia	11	55.76	476.95	–	–
Megali Prespa	12	61.62	412.20	2.34	0.74
Mikri Prespa	29	55.50	321.41	2.86	0.62
Ozeros	13	61.54	507.89	1.82	0.55
Pamvotida	9	53.24	367.58	–	–
Petron	12	54.80	460.99	2.02	0.55
Pikrolimni	11	49.09	367.86	2.13	0.64
Piniou	8	62.50	389.38	2.25	0.80
Stymfalia	15	53.65	153.34	1.63	0.43
Tavropos	15	50.63	248.27	0.94	0.36
Trichonida	11	63.94	320.84	1.84	0.53
Vegoritida	15	53.97	362.56	1.26	0.36
Volvi	30	56.90	350.91	2.07	0.54
Voulkaria	19	53.70	288.43	1.64	0.39
Yliki	17	53.31	342.40	1.30	0.34
Zazari	31	57.03	338.94	–	–

– Indicate lakes where species abundance data were not available

with any parameter and did not differentiate among the categories conductivity and trophic state (Fig. 5). For J', significant differences were recorded only between the categories of trophic state ($H = 8.53$, $P < 0.05$) (Fig. 6).

The randomization test applied to Δ^+ showed that even though many lakes had values below the theoretical mean, the majority fell within the 95% probability funnel (Fig. 7a). However, Lakes Karla and Koronia had lower community distinctness than

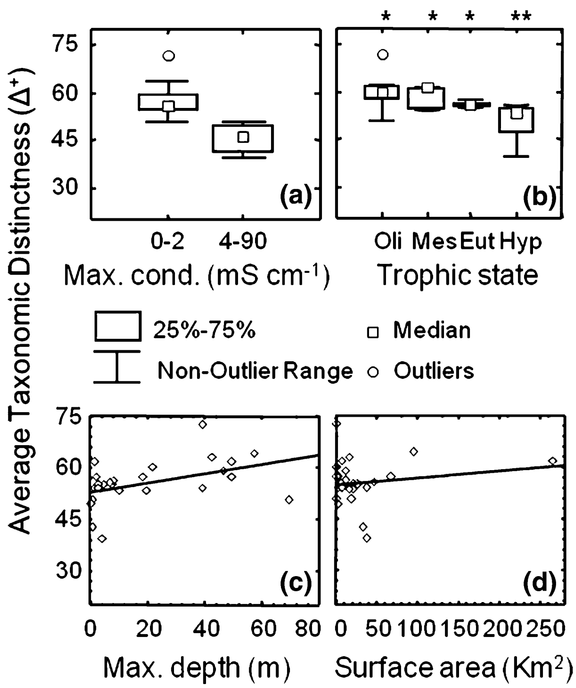

Fig. 3 *Box plots* of Average Taxonomic Distinctness (Δ^+) for rotifer species lists of the 26 Greek lakes grouped by (**a**) salinity ($P < 0.0001$) and (**b**) trophic state, *Oli* oligotrophic, *Mes* mesotrophic, *Eu* eutrophic and *Hyp* hypereutrophic ($P < 0.0001$). *, ** Significant differences (Bonferroni test). Scatter plot of Average Taxonomic Distinctness (Δ^+) per lake (*open diamond*) against (**c**) the maximum depth and (**d**) surface area. The relationships were fitted with linear regressions $\Delta^+ = 52.7212 + 0.1355$ maximum depth ($R^2 = 0.20$, $P < 0.05$) and $\Delta^+ = 52.835 + 0.0201$ surface area ($R^2 = 0.03$, $P = 0.43$), respectively

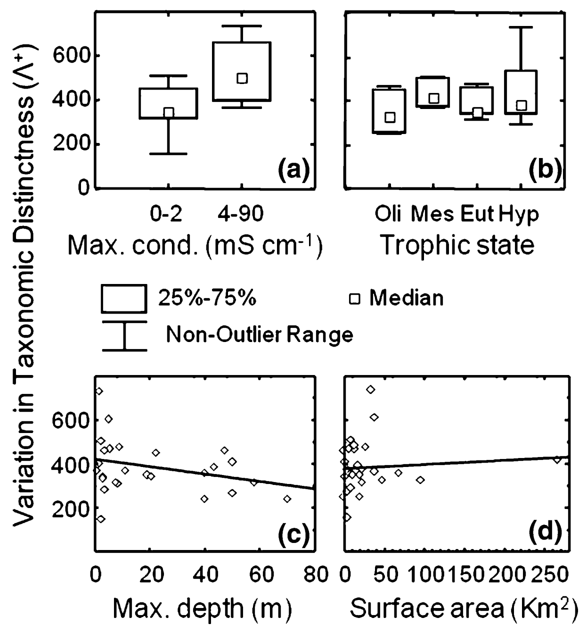

Fig. 4 *Box plots* of Variation in Taxonomic Distinctness (Λ^+) for rotifer species lists of the 26 Greek lakes grouped by (**a**) salinity ($P < 0.05$) and (**b**) trophic state, *Oli* oligotrophic, *Mes* mesotrophic, *Eu* eutrophic and *Hyp* hypereutrophic ($P = 0.32$). Scatter plot of Variation in Taxonomic Distinctness (Λ^+) per lake (*open diamond*), against (**c**) the maximum depth and (**d**) surface area. The relationships were fitted with linear regressions $\Lambda^+ = 418.9108 + 1.6745$ maximum depth ($R^2 = 0.09$, $P = 0.13$) and $\Lambda^+ = 3799.3283 + 0.1838$ surface area ($R^2 = 0.01$, $P = 0.69$), respectively

expected from the general taxonomic relationships in the species pool. Furthermore, the low values of Δ^+ recorded for Lakes Karla and Koronia, and for Lakes Ismarida and Pikrolimni, were accompanied by a lower number of rotifer families compared to their species richness (Fig. 8).

For Λ^+ the randomization test showed that all lakes had higher values than the lower 95% limit of the probability funnel. However, eight lakes (Amvrakia, Doirani, Ismarida, Karla, Koronia, Lysimachia, Ozeros and Petron) were placed above the funnel (Fig. 7b). For these lakes, the rotifer community was overrepresented by species of the family Brachionidae, which had a more than 40% contribution to the species richness of the above lakes (Fig. 9).

When the randomization test for Δ^+ and Λ^+ was performed using the different master species lists, the same lakes were identified as shown in Figs. I and II (Online Resource 3—Supplementary materials). When the randomization test was applied for Δ^+ based on a taxonomic tree with five taxonomic levels, all lakes were placed within the probability funnel (Fig. III, Online Resource 3—Supplementary materials).

Discussion

Rotifer diversity is known to be influenced by a range of factors such as depth, salinity, surface area and trophic state (e.g. Green & Mengestou, 1991; Ejsmont-Karabin, 1995; Allen et al., 1999). We examined these in relation to taxonomic indices. All factors except from surface area showed significant differences for Δ^+. Nevertheless, the lowest values of Δ^+ were recorded for Lakes Ismarida, Karla, Koronia and Pikrolimni, which are characterized by periods of increased salinity (Table 1). Salinity influences rotifer

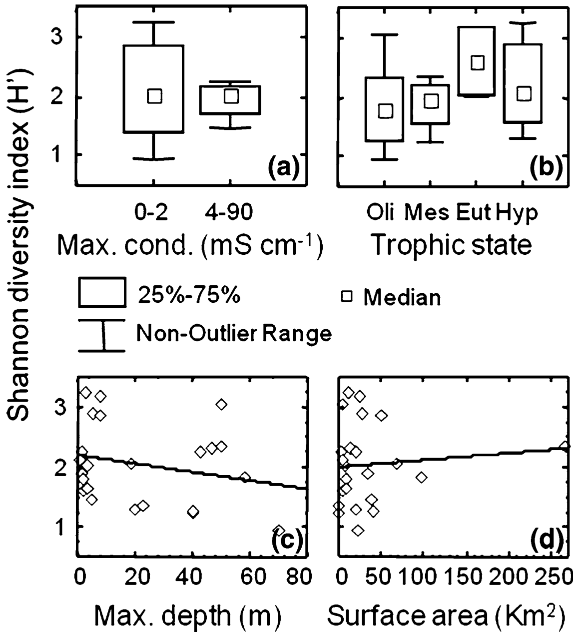

Fig. 5 *Box plots* of Shannon index (H') for rotifer samples of the 23 Greek lakes grouped by (**a**) salinity ($P = 0.83$) and (**b**) trophic state, *Oli* oligotrophic, *Mes* mesotrophic, *Eu* eutrophic and *Hyp* hypereutrophic ($P = 0.23$). Scatter plot of Shannon index (H') per lake (*open diamond*), against (**c**) the maximum depth and (**d**) surface area. The relationships were fitted with linear regressions $H' = 2.1963 - 0.0066$ maximum depth ($R^2 = 0.05$, $P = 0.28$) and $H' = 2.0089 + 0.0011$ surface area ($R^2 = 0.008$, $P = 0.09$), respectively

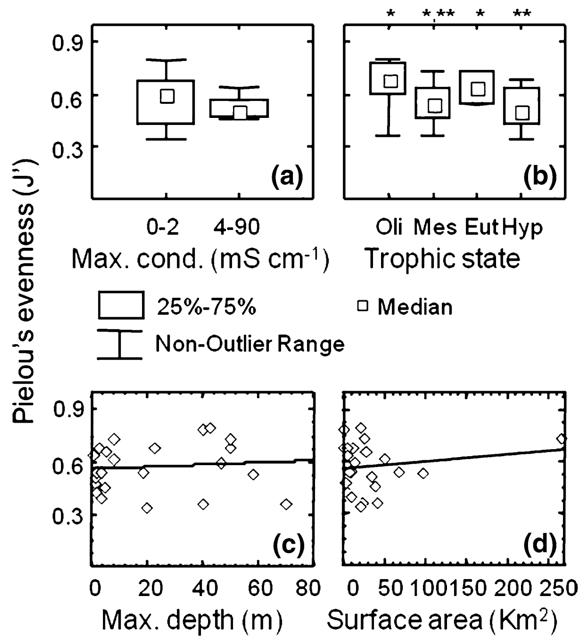

Fig. 6 *Box plots* of Pielou's evenness (J') for rotifer samples of the 23 Greek lakes grouped by (**a**) salinity ($P = 0.20$) and (**b**) trophic state, *Oli* oligotrophic, *Mes* mesotrophic, *Eu* eutrophic and *Hyp* hypereutrophic ($P < 0.05$). *, ** Significant differences (Bonferroni test). Scatter plot of Pielou's evenness (J') per lake (*open diamond*), against (**c**) the maximum depth and (**d**) surface area. The relationships were fitted with linear regressions $J' = 0.5581 + 0.0006$ maximum depth ($R^2 = 0.009$, $P = 0.67$) and $J' = 0.4465 + 0.0004$ surface area ($R^2 = 0.028$, $P = 0.45$), respectively

assemblages, with different species being found in salt lakes with different level of salinity and with different anion dominance (e.g. salt lakes with chloride, sulphate or carbonate-dominated water) (Hammer, 1993; Derry et al., 2003). It is recognized that salinity affects rotifer community structure because increased salinity leads to decreased biodiversity (e.g. Sládeček, 1983; Athibai et al., 2013). Nevertheless, the lowest values of Δ^+ did not reflect lower species diversity in terms of species richness. Instead, they indicated less diverse communities with more closely related species, as reflected in the reduced number of families recorded in comparison to lakes with similar species richness. Apart from identifying communities from lakes with incidence of increased salinity, the randomization test, which discriminates anthropogenically perturbed lakes, differentiated Lakes Karla and Koronia, which have high conductivity due to anthropogenic interventions (Michaloudi et al., 2012; Papadimitriou et al., 2013). In contrast, Lake Pikrolimni as a natron lake (Dotsika et al., 2009) and Lake Ismarida, which has interactions with seawater (Moustaka-Gouni et al., 2011), have high conductivity due to natural processes and fell within the 95% probability funnel. Thus, Δ^+ applied on rotifer communities in lakes confirms the hypothesis that the species present in any lake represent a random selection from the master species list except in the anthropogenically perturbed environments.

The lakes with the increased salinity that were identified by the randomization test of Δ^+ had also the highest values of Λ^+ that differed significantly between groups based on salinity. Lakes with high Λ^+ values were placed outside of the 95% probability funnel reflecting the unevenness of the taxonomic structure due to overrepresentation of taxa from the family Brachionidae, mainly of the genera *Brachionus* and *Keratella*. These lakes [Amvrakia (Dafis et al., 1997), Doirani (Myronidis et al., 2012), Ismarida

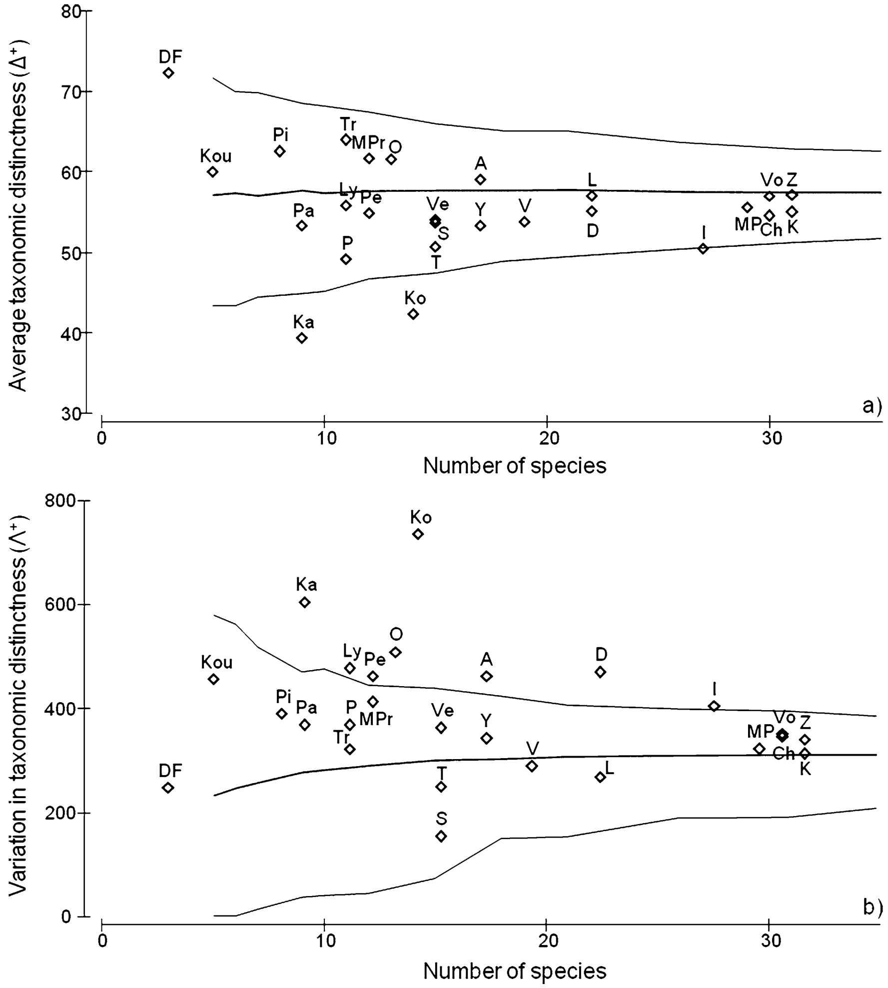

Fig. 7 The randomization test for (**a**) Average Taxonomic Distinctness (Δ^+) and (**b**) Variation in Taxonomic Distinctness (Λ^+) against number of species for rotifer assemblages from 26 Greek lakes. *Central line* is the mean value for the master species list. *Funnel lines* are confidence limits within which 95% of simulated values lie. Abbreviations based on Fig. 1

(Moustaka-Gouni et al., 2011), Karla (Papadimitriou et al., 2013), Koronia (Michaloudi et al., 2012), Lysimachia (Dafis et al., 1997), Ozeros (Dafis et al., 1997) and Petron (Dimitrakopoulos & Koumantakis, 2008)] are characterized by increased water-level fluctuations, mainly due to unsustainable water management. Similarly, a dominance of Brachionidae taxa was also found in Lakes Pikrolimni, Vegoritida and Yliki, which also experience water-level fluctuations (Koussis et al., 2002; Dimitrakopoulos & Koumantakis, 2008; Dotsika et al., 2009). However, Λ^+ failed to differentiate them because these lakes fell inside of the 95% probability funnel. Data on the hydrological aspects of these lakes could probably have helped interpret the above differentiation. Hydrological aspects such as water-level fluctuation and water residence time have been found to influence zooplankton assemblage patterns (Geraldes & Boavida, 2007; Obertegger et al., 2007), although the effects of these parameters on zooplankton communities are not well studied (Leira & Cantonati, 2008). In our study, the result was an overrepresentation of closely related species (i.e. species of the Brachionidae family). Similar results have been found in different studies (e.g. Casanova et al., 2009; Nova et al., 2014), with different species of the genus *Brachionus* being predominant in both low and high water phases (Chaparro et al., 2011).

The analyses performed using the traditional diversity indices revealed statistically significant differences only among the categories of trophic state for J'. The information conveyed by these indices is in terms of abundance, indicating for H' the dominance of one or more species and for J' the equitability of

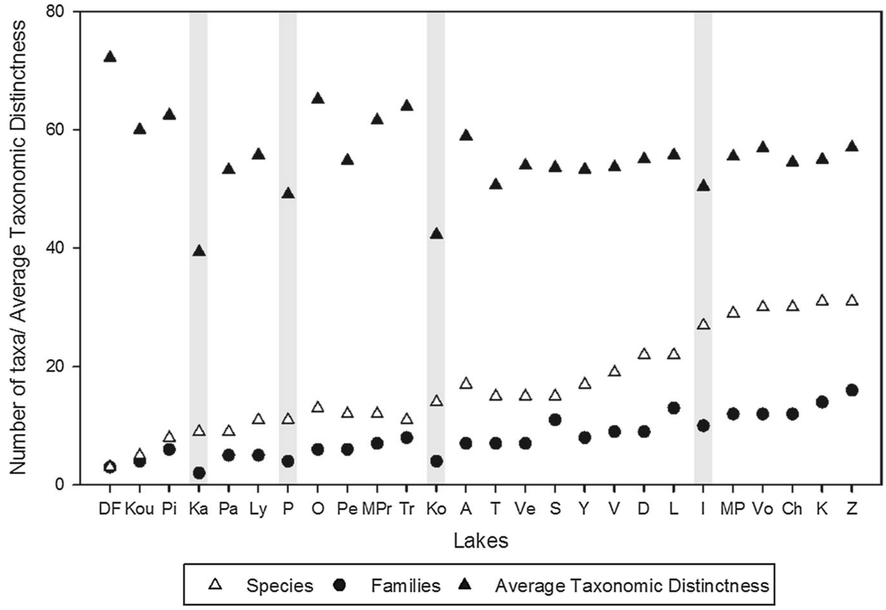

Fig. 8 Average Taxonomic Distinctness (*dark triangle*) against number of species (*open triangle*) and families (*dark circle*) for rotifer assemblages from 26 Greek lakes. The *grey areas* indicate the lakes with the lowest Δ^+ values. Abbreviations based on Fig. 1

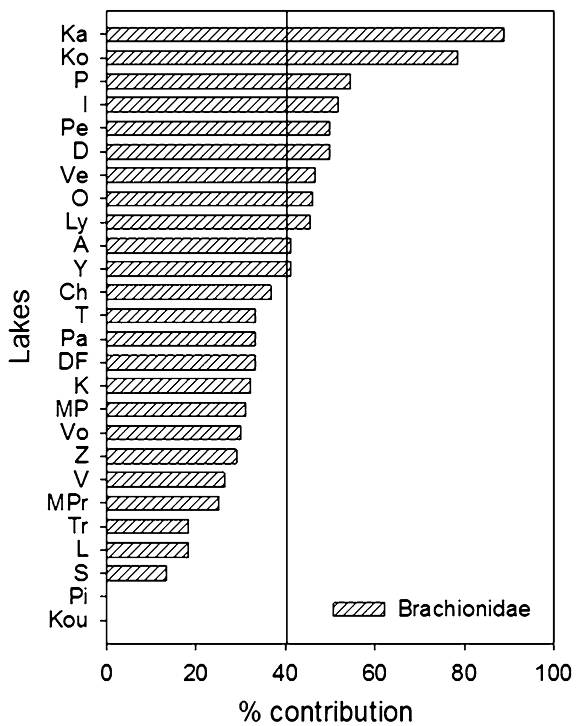

Fig. 9 Percentage (%) contribution of family Brachionidae in the rotifer assemblages of the 26 Greek lakes. Abbreviations based on Fig. 1

their distribution in the community as a result of the environmental variable (Magurran, 2004). The taxonomic relatedness between species is not taken into consideration, while at the same time H' was affected by the number of species. J' did not show any correlation with the number of species which should be expected since evenness as a diversity index was built to be independent from species richness (Gosselin, 2006). It is known that H' is a diversity index depended on sampling effort that can be expressed as number of species, number of samples, number of individuals counted or collected (Warwick & Clarke, 2001; Magurran, 2004). In any case the fact that these indices are based on quantitative data and are thus depended on sampling methodologies does not allow the use of existing data or for comparison of species lists from different regions, and across different methodologies and sampling effort (Clarke & Warwick, 1998; Magurran, 2004). Furthermore, traditional diversity indices do not have a statistical framework, such as the randomization test to differentiate a region based on expected diversity (Leonard et al., 2006). Nevertheless, for the taxonomic distinctness indices identification should be done down to species level in order to identify deviation from the expected biodiversity pattern.

In this study, we have shown that taxonomic distinctness indices applied to rotifer communities can identify disturbed lakes based on the taxonomic relatedness of the species present. Based on the randomization test, lakes with different taxonomic diversity than expected are influenced by increased salinity or water-level fluctuation. Thus, taxonomic distinctness indices based on rotifers may prove to be a useful tool in ecosystem monitoring, identifying lake disturbance in an easy and cost-effective way considering that they are insensitive to sampling effort and easy to measure because they rely on presence/absence data, only, and rotifers are easy and inexpensive to sampling. Our conclusions need to be tested further across a wider range of lakes because of the differences that are likely to occur across geographical regions.

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