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ROTIFERA XV

Trophic state assessment based on zooplankton communities in Mediterranean lakes

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Abstract Eutrophication assessment is made widely using Carlson Trophic State indices (TSI) [e.g. secchi disc depth (TSI_{SD})] or phytoplankton biomass. Recently, two Carlson type indices using rotifers (TSI_{ROT}) and crustaceans (TSI_{CR}) were developed from Polish lakes. In the present study, both indices were applied to zooplankton communities from 16 Greek lakes, covering the entire trophic state spectrum, in order to test their application in a different climatic zone, the Mediterranean. The evaluation of the indices (TSI_{ROT} and TSI_{CR}) was made comparing the trophic state of each sampling/lake based on TSI_{SD} and mean summer phytoplankton biomass. Both indices increased across the eutrophication gradient but misclassify the trophic state. We propose a new index, TSI_{ZOO}, the average of the formulae TSI_{ROT} and TSI_{CR} which are significantly correlated with the eutrophication proxies. All three zooplanktonic indices can efficiently detect low (oligotrophic–mesotrophic) and high (eutrophic–hypertrophic) trophic state using the boundaries < 45 for TSI_{ROT} and TSI_{ZOO} and < 50 for TSI_{CR}. All zooplanktonic indices are promising and effective tools for monitoring and assessment of eutrophication of Mediterranean lakes when mean values are used. Still, TSI_{ZOO} should be preferred as the best index that correlated with eutrophication which had the best estimations.

Keywords Carlson Trophic State indices · TSI_{CR} · TSI_{ZOO} · TSI_{ROT} · Greek lakes · Eutrophication

Introduction

The increase of human population and the expansion of agricultural and industrial activities have led to anthropogenic eutrophication and deterioration of the health of freshwater systems worldwide (Callisto et al., 2014). One of the first and most widely used classification schemes developed for lake trophic state assessment is Carlson Trophic State Index (TSI)

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(Carlson, 1977) based on Secchi depth (SD), total phosphorus (TP) and the concentration of the phytoplankton pigment chlorophyll *a* (chl *a*). SD is an easy and low-cost variable of measuring water transparency, thus assessing lake water quality; still, it is influenced both by phytoplankton abundance and non-algal particulate matter (Carlson, 1977). Phytoplankton is considered one of the most appropriate elements for lake trophic state assessment due to its short generation times and direct responses to changing nutrient conditions (both total phosphorus and nitrogen) (Lyche-Solheim et al., 2013). Chl *a* as a proxy of phytoplankton biomass has been widely adopted in water quality assessments of national (e.g. Carlson, 1977; Wolfram et al., 2009), European (EC, 2008) and worldwide (OECD, 1982) lake monitoring and classification schemes. Nevertheless, its use as an indicator of biomass is questionable (Kruskopf & Flynn, 2006) because chl *a* concentration can vary considerably depending on algal composition, their physiological state and to a lesser extent on light (Reynolds, 1984; Moustaka-Gouni, 1989). Algal biomass estimation, being though a more laborious procedure that requires direct counts and measurements, is the most accurate and fundamental measurement of phytoplankton, the critical metric related to eutrophication and water quality (e.g. Katsiapi et al., 2016). Consequently, phytoplankton metrics can be successfully used in lake monitoring and trophic state assessments especially when eutrophication pressure is considered (Katsiapi et al., 2016). However, there are cases, as for example shallow macrophyte-dominated lakes, where top–down control by zooplankton grazers may limit phytoplankton biomass (Jeppesen et al., 1997), where other components of the lake food web should also be considered critical. Moreover, since both the structure and the function of the food web changes across the eutrophication gradient (Havens, 2014), there is a need for a holistic approach towards this direction. Even though zooplankton has been neglected as a biological quality element (BQE) in the Water Framework Directive 2000/60/EE, the Common Implementation Strategy (Guidance document 23) indicates "Zooplankton grazing (top–down control) which may be influenced by other anthropogenic activities" as an additional lake specific—supporting environmental factor featuring the eutrophication impact; zooplankton has also been included in the checklist for a holistic lake assessment (CIS, 2009). Recently, two Carlson type indices have been developed using zooplankton data from Polish lakes, TSI_{ROT} based on rotifer communities (Ejsmont-Karabin, 2012) and TSI_{CR} based on crustacean (cladocerans and copepods) communities (Ejsmont-Karabin & Karabin, 2013).

Zooplankton communities can respond quickly to changes resulting by trophic cascades either through bottom–up or top–down control (Carpenter et al., 1985). Thus, they have been used not only in trophic state assessment (Ejsmont-Karabin, 2012; Ejsmont-Karabin & Karabin 2013) but also in aquatic ecotoxicology (e.g. Sarma & Nandini, 2006; Snell & Joaquim-Justo, 2007; Kulkarni et al., 2013), and in providing information about water quality (Azémar et al., 2010; Haberman & Haldna, 2014). Crustaceans have even been included in recently developed multimetric indices for ecological water quality assessment (Moss et al., 2003; Kane et al., 2009). Regarding trophic state assessment, studies such as Pejler (1983) and Karabin (1985) have shown that zooplankton communities tend to have a higher abundance and biomass and increased contributions of bacterivorous rotifers and cyclopoids following an increase in trophic state. Moreover, some taxa begin to dominate the communities, while other decrease in average body weight. However, it should be taken into consideration that these patterns have been described mainly by the well-studied cold-temperate European lakes and might not apply in different climatic zones. Mediterranean lakes have been differentiated from temperate lakes by morphometric (basin size/lake size) and climatic characteristics (Alvarez Cobelas et al., 2005). Further biologically important differences are related to the increased availability of solar radiation in winter months leading to the continuous increase of phytoplankton mainly during late-autumn and winter months and to an extended period of fish reproduction resulting in more persistent fish predation on zooplankton (Moustaka-Gouni et al., 2014). Thus, patterns developed based on temperate lakes might be differentiated as the Plankton Ecology Group model (PEG-model) does (Moustaka-Gouni et al., 2014). Based on the Mediterranean PEG-model, it seems that zooplankton communities cannot effectively graze phytoplankton due to both high phytoplankton crops and fish predation. However, zooplankton communities still play an important role in lake assessment in the Mediterranean region, since rotifers have been used for discriminating anthropogenically disturbed lakes using taxonomic distinctness indices (Stamou et al., 2017) and crustaceans have been used for estimating the water quality of wetlands using QAELS index (Boix et al., 2005).

Based on the above differentiations of Mediterranean waters, we hypothesised that trophic state indices developed for temperate lakes, TSI_{ROT} and TSI_{CR}, might differentiate and may need adaptions when applied in a different climatic zone, in our case the Mediterranean. In order to test the above hypothesis, we evaluated their application in 16 Greek lakes, along the entire trophic spectrum, a wide range of altitude, surface area, mean and maximum depth. The evaluation of these indices was made using the trophic state estimated by the respective TSI_{SD} and mean summer phytoplankton biomass. Furthermore, we tested the dependance of each metric/formula of TSI_{ROT} and TSI_{CR}, on eutrophication and propose a new TSI index based on the whole zooplankton community, namely TSI_{ZOO}, combining, in part, both TSI_{ROT} and TSI_{CR}, as a zooplanktonic index for detecting eutrophication in Mediterranean lakes. Finally, we tested which of the three zooplanktonic indices (TSI_{ROT}, TSI_{CR} and TSI_{ZOO}) is more efficient in discriminating the trophic state in Mediterranean region.

Materials and methods

Data collection

In our analysis, we used data from 16 Greek lakes; two of them, Kremasta and Tavropos, are reservoirs whereas the rest are natural lakes (Fig. 1). The 16 lakes encompass a wide range of altitude, surface area, mean and maximum depth (Table 1) and cover the entire trophic state spectrum. The dataset we used comprises published (from 1984 to 2016) and new data (from 2004 and 2016 to 2017) of the summer period (3 samplings during the period June to September) (Online Resource 1, Table I). The summer period was chosen because it was used for the development of TSI_{ROT} and TSI_{CR} but also because this is the period used for the lake ecological status assessment in Greece/Mediterranean region (e.g. Pahissa et al., 2015; Katsiapi et al., 2016; Petriki et al., 2017). The number of samples per lake ranged from 3 to 12 (Online Resource 1, Table I). The same sampling protocol for phytoplankton and zooplankton was followed for all lakes and it is well described by Mazaris et al. (2010) and Moustaka-Gouni et al. (2014). Secchi depth (SD) was also measured during the samplings.

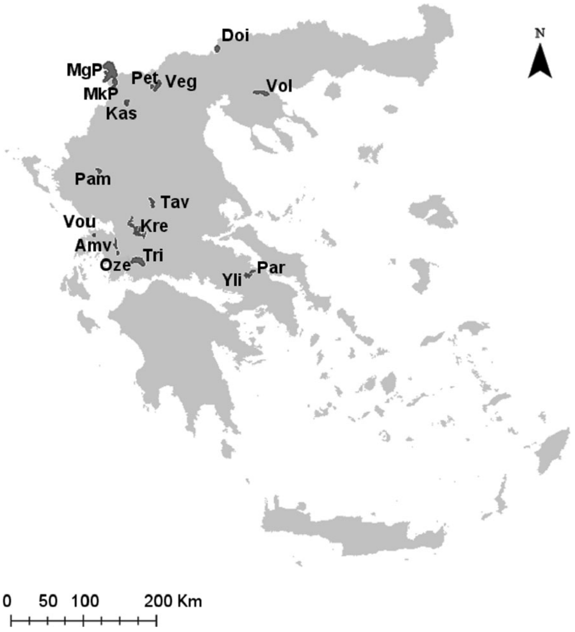

Fig. 1 Map of Greece showing the locations of the 16 lakes included in the study. *Amv* Amvrakia, *Doi* Doirani , *Kas* Kastorias , *Kre* Kremasta , *MgP* Megali Prespa , *MkP* Mikri Prespa , *Oze* Ozeros , *Pam* Pamvotis , *Par* Paralimni , *Pet* Petron , *Tav* Tavropos , *Tri* Trichonis , *Veg* Vegoritis , *Vol* Volvi, *Vou* Voulkaria , *Yli* Yliki

Zooplankton analysis

For zooplankton samples analysis, the lowest possible taxonomic level (genus or species) was identified using the taxonomic keys of Koste (1978), Nogrady et al. (1995), Segers (1995), Ricci & Melone (2000) and Nogrady & Segers (2002) for rotifers except Bdelloidea; of Amoros (1984), Korovchinsky (1992), Alonso (1996) and Benzie (2005) for cladocerans and of Dussart (1967a, b), Kiefer (1968, 1971), Reddy (1994), Einsle (1996) and Dussart & Defaye (2001) for copepods.

All lines of taxonomic information (i.e. spellings, valid names) were confirmed using the Rotifer World Catalog (Jersabek & Leitner, 2013) and the List of Available Names (LAN) part Rotifera (Segers et al.,

Table 1 Topographic, morphological data and the type of mixing for the 16 studied Greek lakes

Lake	Latitude	Longitude	Surface area (Km^2)	Altitude (m)	Mean depth (m)	Max depth (m)	Mixing type[a]
Amvrakia	38°45′15.60″	21°10′55.09″	14.5	25	22	53	W.M.
Doirani	41°12′55.52″	22°44′48.34″	34.8	142	3	8	P.
Kastorias	40°31′09.59″	21°17′36.13″	30.0	629	4	9	P.
Kremasta	38°52′57.40″	21°36′04.60″	71.7	267	47	92	W.M.[b]
Megali Prespa	40°52′03.87″	21°01′29.31″	256.8	844	18	55	W.M.
Mikri Prespa	40°46′22.80″	21°05′05.96″	39.2	850	4	9	P.
Ozeros	38°39′16.81″	21°13′32.40″	9.4	24	4.5	5.5	P.
Pamvotis	39°39′45.57″	20°53′27.68″	22.0	470	4.3	9.2	P.
Paralimni	38°27′53.7″	23°20′55.5″	10.0	51	4	8	P.
Petron	40°43′38.79″	21°41′49.60″	11.0	572	3	6	P.
Tavropos	39°17′39.60″	21°45′9.28″	21.6	800	> 15	60	W.M.[b]
Trichonis	38°32′47.1″	21°35′12.7″	97.2	16	30	59	W.M.
Vegoritis	40°45′11.06″	21°47′13.76″	46.0	524	25	52	W.M.
Volvi	40°40′37.57″	23°28′50.61″	68.6	37	13	28	W.M.
Voulkaria	38°52′12.8″	20°50′24.2″	9.4	5	1.6	2.5	P.
Yliki	38°23′56.77″	23°16′19.38	23.0	78	21	34	W.M.

[a] W.M.: warm monomictic and P.: polymictic type

[b] Even though they show thermal stratification, they are influenced by water abstractions from hydroelectric power plants

2012; International Commission on Zoological Nomenclature: http://iczn.org/lan/rotifer), the cladoceran checklist (Kotov et al., 2013) and the World of Copepods database (Walter & Boxshall, 2018). Abundance estimation (expressed as ind/l) was performed following the method of Bottrell et al. (1976), Downing & Rigler (1984) and Taggart (1984). For each sample (total volume of 100 or 50 ml), five counts of 1 ml subsamples were made on a Sedgewick-Rafter cell. At least 300 individuals were counted or all the individuals were counted. For wet biomass estimations (expressed as mg/l), individual geometric formulae (Ruttner-Kolisko, 1977) or dry weight data and length–weight regressions were used (Dumont et al., 1975; Michaloudi, 2005); dry weight was consequently transformed to wet weight assuming that dry weight is 10% of wet (Dumont et al., 1975).

Phytoplankton analysis

Phytoplankton samples, live and preserved in Lugol's solution, were examined in sedimentation chambers under a light inverted microscope; species were identified using appropriate taxonomic keys (e.g. Huber-Pestalozzi, 1938; Tikkanen, 1986).

Phytoplankton counts were performed using Utermöhl's (1958) sedimentation method. For biovolume estimation, the dimensions of 30 individuals (cells, filaments or colonies) of each species were measured using tools of a digital microscope camera (Nikon DS-L1, software: DS camera control unit DS-L1) and the mean cell or filament volume estimates were calculated using appropriate geometric formulae (Hillebrand et al., 1999). Biovolume converted to biomass values using a density of 1 g/cm^3.

Trophic state indices

For the assessment of lakes trophic state, we applied four trophic state indices. The TSI index based on Secchi depth (TSI_{SD}), the mean summer phytoplankton biomass and the TSI indices based on zooplankton communities, the Rotifer Trophic State index (TSI_{ROT}) and the Crustacean Trophic State index (TSI_{CR}).

TSI_{SD} was estimated based on the Secchi depth (SD) (Eq. 1) (Carlson, 1977) measured during each zooplankton–phytoplankton sampling; the classification was based on the modified boundaries of Carlson & Simpson (1996).

$$TSI_{SD} = 60 - 14.41\ \ln(SD) \qquad (1)$$

The mean summer phytoplankton biomass of the respective zooplankton–phytoplankton samplings was used to determine the lakes' trophic state according to the following classification schemes: (a) Smith (2003) for natural oligotrophic lakes with mean depth > 15 m at altitude > 400 m and (b) Wetzel (2001) for the rest of the lakes.

Both TSI_{ROT} and TSI_{CR} are described based on data of Polish lakes as the average value of 6 formulae (Table 2). For both indices, the boundaries are set as $TSI < 45$ mesotrophic, $45 < TSI < 55$ meso-eutrophic, $55 < TSI < 65$ eutrophic and $TSI > 65$ hypertrophic (Ejsmont-Karabin, 2012; Ejsmont-Karabin & Karabin, 2013).

In the present study, we estimated TSI_{ROT} based on abundance and wet biomass data of rotifer communities of 16 Greek lakes, as the mean value of the TSI_{ROT1}, TSI_{ROT2}, TSI_{ROT3} and TSI_{ROT4} formulae (Eqs. 2–5, Table 2). We did not use the formula for the percentage of *tecta* form in the *Keratella cochlearis* (Gosse, 1851) population (Eq. 6, Table 2) since in our dataset the abundance of *Keratella tecta* (Gosse, 1851) was not always recorded separately from *K. cochlearis* since until recently *K. tecta* was considered a variation of *K. cochlearis* and not a separate species in the *Keratella cochlearis* species complex (Cieplinski et al., 2017). The TSI_{ROT6} formula of the indicative of high trophic state rotifers (Eq. 7, Table 2) was not used since the indicator species are not appropriate for Greek lakes. For example, *Ascomorpha ecuadis* (Perty, 1850) and *Gastropus stylifer* (Imhof, 1891), which were used by Ejsmont-Karabin (2012) as indicators of low trophic state, were also found in eutrophic even hypertrophic Greek lakes (Yliki, Mikri Prespa, Trichonis) and *Pompholyx sulcata* (Hudson, 1885) an indicator of high trophic state, according to Ejsmont-Karabin (2012), was dominant in the oligotrophic lake Amvrakia (Online Resource 2). Based on the above data, it was evident that this formula could not be used for Greek lakes.

TSI_{CR} was also estimated based on abundance and wet biomass data of crustacean (cladocerans and copepods) communities of 16 Greek lakes, as the mean value of the TSI_{CR1}, TSI_{CR2}, TSI_{CR3} and TSI_{CR4} formulae (Eqs. 8–11, Table 2). We did not use the formula TSI_{CR5} (Eq. 12, Table 2) for the ratio of cyclopoid to calanoid biomass since it was developed only for dimictic lakes and the studied lakes were polymictic and warm monomictic (Table 1). The TSI_{CR6} formula (Eq. 13, Table 2) of the indicator crustaceans of high trophic state was also not used. The species *Heterocope appendiculata* (Sars G.O. 1863), *Bosmina (Eubosmina) coregoni* (Baird, 1857) and *Bythotrephes longimanus* (Leydig, 1860) which were proposed as indicators of low trophic state by Ejsmont-Karabin & Karabin (2013) have not been recorded in the studied Greek lakes while others, i.e. *Daphnia (Daphnia) cucullata* (Sars, 1862) (indicative of low trophic state) and *Bosmina (Bosmina). longirostris* (O. F. Müller, 1776) (indicative of high trophic state) have been recorded in the entire trophic spectrum in the studied Greek lakes indicating the unsuitability of the indicators species formula (Online Resource 2).

Statistical analysis

Linear regression and Analysis of Variance (ANOVA) were applied to test the dependence of each one of the TSI_{ROT} ($TSI_{ROT1} - TSI_{ROT4}$) and TSI_{CR} ($TSI_{CR1} - TSI_{CR4}$) formulae on eutrophication as estimated with the use of two proxies, TSI_{SD} and phytoplankton biomass.

Based on the above analyses, we propose a new index, the Zooplankton Trophic State Index (hereafter called TSI_{ZOO}) using only the mean value of the formulae, which were significantly correlated with eutrophication. The boundaries for TSI_{ZOO} used for lakes classification were the same used for the TSI_{ROT} and TSI_{CR} indices, i.e. $TSI < 45$ mesotrophic, $45 < TSI < 55$ meso-eutrophic, $55 < TSI < 65$ eutrophic and $TSI > 65$ hypertrophic.

Then, linear regression and ANOVA were applied to test the best-fitted trophic index based on zooplankton data, namely TSI_{ROT} (the average of $TSI_{ROT1} - TSI_{ROT4}$), TSI_{CR} (the average of $TSI_{CR1} - TSI_{CR4}$) and TSI_{ZOO} (the average of TSI_{ROT1}, TSI_{ROT2}, TSI_{CR1} and TSI_{CR2}) compared to the two proxies of eutrophication. The same analyses were also applied to test the dependence of the TSI_{ROT}, TSI_{CR} and TSI_{ZOO} indices on the percentage contribution of cyanobacteria to total phytoplankton biomass since the Mediterranean

Table 2 The equations of TSI_{ROT} and TSI_{CR} developed for Polish lakes by Ejsmont-Karabin (2012) and Ejsmont-Karabin & Karabin (2013), respectively

Equations	Abbreviations	Comments
$TSI_{ROT1} = 5.38Ln(N) + 19.28$ (2)	N: total rotifera abundance (ind/l)	
$TSI_{ROT2} = 5.63Ln(B) + 64.47$ (3)	B: total wet rotifera biomass (mg/l) excluding *Asplanchna* spp.	*Asplanchna* spp. Gosse, 1850 was excluded due to its larger body size compared to other rotifers
$TSI_{ROT3} = 0.23BAC + 44.30$ (4)	BAC: the percentage of the bacterivorus rotifers in terms of rotifer abundance	*Anuraeopsis fissa* (Gosse, 1851), *Filinia* spp. Bory de St. Vincent, 1824, *Brachionus angularis* Gosse, 1851, *Keratella cochlearis* (Gosse, 1851), *Pompholyx sulcata* Hudson, 1885
$TSI_{ROT4} = 3.85(B : N)^{-0.318}$ (5)	B:N: the ratio of wet biomass to rotifera abundance (mg/ind)	
$TSI_{ROT5} = 0.187\ TECTA + 50.38$ (6)	TECTA: the percentage of *Keratella cochlearis* f. *tecta* individuals to the sum of *K. cochlearis* population	
$TSI_{ROT6} = 0.203IHT + 40.0$ (7)	IHT the percentage of species indicators of high trophic state to sum of species indicators	Indicators of high trophic state: *A. fissa*, *B. angularis*, *Brachionus calyciflorus* Pallas, 1766, *Filinia longiseta* (Ehrenberg, 1834), *K. cochlearis* f. *tecta* (Gosse, 1851), *Keratella quadrata* (Müller, 1786), *P. sulcata* and *Trichocerca pusilla* (Jennings, 1903) Indicators of low trophic state: *Ascomorpha ecaudis* Perty, 1850, *Ascomorpha ovalis* (Bergendal, 1892) *Gastropus stylifer* Imhof, 1891.
$TSI_{CR1} = 25.5N^{0.142}$ (8)	N: total crustacean abundance (ind/l)	
$TSI_{CR2} = 57.6B^{0.081}$ (9)	B: total wet cyclopoids biomass (mg/l)	
$TSI_{CR3} = 40.9CB^{0.097}$ (10)	CB: Percentage of cyclopoid biomass in the total crustacean biomass	
$TSI_{CR4} = 58.3(CY/CL)^{0.071}$ (11)	CY/CL: ratio of cyclopoids to cladocerans biomass	
$TSI_{CR5} = 5.08\ Ln(CY/CA) + 46.6$ (12)	CY/CA: ratio of cyclopoids to calanoids biomass	
$TSI_{CR6} = 43.8\ e^{0.004(IHT)}$ (13)	IHT the percentage of species indicators of high trophic state to sum of species indicators	Indicators of high trophic state: *Mesocyclops leuckarti leuckarti* (Claus, 1857), *Thermocyclops oithonoides* (Sars G.O., 1863), *Diaphanosoma brachyurum* (Liévin, 1848), *Chydorus sphaericus* (O. F. Müller, 1776), *Bosmina (Eubosmina) coregoni* Baird, 1857 [Syn: Bosmina (E.) coregoni thersites (Poppe, 1887)], *Bosmina (Bosmina) longirostris* (O. F. Müller, 1776) Indicators of low trophic state: *Heterocope appendiculata* Sars G.O., 1863, *Bosmina (Eubosmina) coregoni* Baird, 1857 Syn.: *Bosmina berolinensis* Imhof, 1888), *Bythotrephes longimanus* Leydig, 1860, *Daphnia (Daphnia) galeata* Sars, 1864, *Daphnia (Daphnia) cristata* Sars, 1862, *Daphnia (Daphnia) cucullata* Sars, 1862

Formulae in bold are those used in the present study

Table 3 Assessment of eutrophication based on the mean values (± standard deviation) of TSI_{SD}, phytoplankton (mean summer) biomass (PB) (mg/l), rotifer communities (TSI_{ROT} and its formulae $TSI_{ROT1} - TSI_{ROT4}$), crustacean communities (TSI_{CR} and its formulae $TSI_{CR1} - TSI_{CR4}$) and zooplankton community (TSI_{ZOO}). Codes are based on lakes abbreviation according to Fig. 1 followed by year of sampling, m.v.: missing value

Codes	TSI_{SD}	PB	TSI_{ROT1}	TSI_{ROT2}	TSI_{ROT3}	TSI_{ROT4}	TSI_{ROT}
Amv_16	33.24 ± 4.01	0.16 ± 0.05	22.82 ± 5.38	26.60 ± 10.04	52.41 ± 2.31	42.28 ± 16.23	36.03 ± 3.40
Doi_04	70.70 ± 5.77	21.04 ± 2.23	57.07 ± 3.20	63.47 ± 2.83	47.10 ± 2.78	39.09 ± 3.47	51.43 ± 2.63
Kas_99	78.31 ± 2.55	13.39 ± 3.48	54.97 ± 0.94	60.19 ± 1.69	52.12 ± 4.33	40.52 ± 3.64	51.95 ± 1.73
Kas_16	m.v.	8.04 ± 6.52	56.12 ± 6.79	60.58 ± 4.54	52.74 ± 6.99	42.60 ± 6.39	53.01 ± 6.01
Kre_16	51.89 ± 2.10	0.44 ± 0.24	35.71 ± 3.29	41.98 ± 2.58	44.84 ± 0.94	35.24 ± 2.38	39.44 ± 2.00
MgP_16	46.72 ± 4.87	1.82 ± 1.96	31.75 ± 9.82	32.34 ± 10.13	53.60 ± 7.57	49.46 ± 2.50	41.79 ± 6.33
MkP_90	64.91 ± 2.18	19.87 ± 11.04	44.22 ± 5.78	47.42 ± 9.60	59.07 ± 3.31	44.58 ± 9.87	48.82 ± 2.40
MkP_91	57.22 ± 1.48	4.08 ± 2.25	46.50 ± 7.08	50.04 ± 2.80	55.40 ± 9.16	44.23 ± 11.08	49.04 ± 6.50
MkP_92	62.10 ± 1.26	5.32 ± 2.07	38.08 ± 2.20	44.92 ± 6.98	49.77 ± 4.56	35.85 ± 10.25	42.16 ± 1.25
MkP_16	53.14 ± 3.11	1.20 ± 0.54	37.24 ± 8.51	37.85 ± 8.54	56.41 ± 2.95	50.06 ± 1.44	45.39 ± 4.99
Oze_16	47.12 ± 8.29	0.40 ± 0.47	50.68 ± 1.26	57.97 ± 8.33	44.30 ± 0	37.38 ± 16.04	47.58 ± 1.76
Pam_16	82.65 ± 9.15	16.44 ± 3.14	45.69 ± 4.00	51.13 ± 4.00	48.93 ± 3.79	38.99 ± 3.68	46.19 ± 0.90
Par_17	60.20 ± 2.93	2.20 ± 1.69	55.81 ± 2.11	63.50 ± 4.49	46.52 ± 2.85	36.23 ± 10.01	50.52 ± 2.33
Pet_10	65.29 ± 3.59	9.83 ± 1.17	54.09 ± 2.49	59.19 ± 3.37	50.89 ± 3.49	40.44 ± 2.17	51.15 ± 1.96
Tav_87	47.43 ± 1.55	0.19 ± 0.10	25.64 ± 4.57	38.26 ± 6.60	45.85 ± 1,29	24.72 ± 2.48	33.62 ± 2.36
Tri_16	24.46 ± 2.18	2.45 ± 1.22	33.38 ± 2.56	43.61 ± 5.35	44.82 ± 0.75	28.89 ± 4.60	37.67 ± 0.89
Veg_87	46.04 ± 0.64	1.90 ± 0.98	26.64 ± 1.52	26.57 ± 3.37	60.07 ± 3.85	51.14 ± 9.56	41.11 ± 2.78
Veg_17	41.28 ± 1.11	2.39 ± 1.89	40.48 ± 4.34	42.19 ± 4.73	45.38 ± 0.53	47.30 ± 3.54	43.84 ± 2.19
Vol_84	54.17 ± 0.96	2.76 ± 0.51	35.85 ± 1.22	46.54 ± 2.91	46.81 ± 3.44	28.29 ± 2.62	39.37 ± 0.52
Vol_85	53.38 ± 0.70	8.33 ± 4.27	42.13 ± 1.24	46.47 ± 3.64	52.58 ± 6.47	41.34 ± 5.68	45.63 ± 1.85
Vol_86	53.01 ± 1.97	4.00 ± 2.01	52.38 ± 3.56	58.97 ± 3.43	49.79 ± 3.18	37.21 ± 2.36	49.56 ± 2.56
Vou_16	76.86 ± 6.62	42.24 ± 28.37	47.16 ± 5.92	50.53 ± 8.44	47.47 ± 0.84	44.31 ± 6.90	47.37 ± 2.26
Yli_90	81.61 ± 2.28	18.66 ± 7.68	51.21 ± 2.44	53.27 ± 5.02	62.65 ± 1.50	49.12 ± 15.52	54.06 ± 3.62

Codes	TSI_{CR1}	TSI_{CR2}	TSI_{CR3}	TSI_{CR4}	TSI_{CR}	TSI_{ZOO}
Amv_16	42.19 ± 2.22	47.05 ± 1.46	52.60 ± 1.98	59.60 ± 4.20	50.36 ± 1.51	34.66 ± 4.08
Doi_04	60.21 ± 3.66	53.83 ± 3.13	55.81 ± 2.57	58.84 ± 4.35	57.17 ± 2.79	58.65 ± 0.42
Kas_99	56.42 ± 6.95	54.21 ± 1.73	58.75 ± 1.97	63.25 ± 5.95	58.16 ± 0.96	56.45 ± 2.73
Kas_16	52.83 ± 4.67	53.11 ± 2.83	58.35 ± 3.39	66.18 ± 12.61	57.62 ± 3.26	55.66 ± 4.42
Kre_16	41.83 ± 1.01	41.58 ± 3.75	53.16 ± 4.24	54.82 ± 2.99	47.85 ± 2.95	40.27 ± 1.28
MgP_16	40.26 ± 5.66	45.65 ± 3.21	53.76 ± 0.13	60.58 ± 2.83	50.06 ± 1.92	37.50 ± 7.15
MkP_90	58.15 ± 1,09	56.55 ± 1.69	56.07 ± 3.10	57.34 ± 3.95	57.03 ± 2.32	51.59 ± 4.47
MkP_91	55.10 ± 3.38	53.39 ± 2.89	54.69 ± 2.44	59.17 ± 0.82	55.59 ± 1.91	51.26 ± 1.65
MkP_92	54.81 ± 1.38	51.70 ± 1.40	52.42 ± 0.97	54.67 ± 1.68	53.40 ± 0.84	47.38 ± 2.76
MkP_16	41.11 ± 5.09	44.69 ± 6.40	53.10 ± 4.61	59.77 ± 2.68	49.67 ± 4.25	40.22 ± 7.03
Oze_16	51.04 ± 8.55	24.85 ± 21.52	25.29 ± 22.15	29.59 ± 25.59	32.67 ± 18.77	46.14 ± 6.83
Pam_16	53.64 ± 9.76	51.85 ± 5.10	54.94 ± 2.17	54.26 ± 2.16	53.67 ± 4.34	50.58 ± 5.84
Par_17	57.13 ± 1.18	54.25 ± 1.89	59.22 ± 1.57	62.76 ± 2.54	58.34 ± 0.93	57.67 ± 0.53
Pet_10	60.16 ± 2.92	56.74 ± 1.71	58.90 ± 2.37	59.38 ± 2.92	58.80 ± 1.24	57.55 ± 0.62
Tav_87	37.88 ± 0.54	47.54 ± 1.36	56.42 ± 2.20	54.67 ± 2.24	49.13 ± 1.32	37.33 ± 3.01
Tri_16	42.54 ± 2.01	31.79 ± 27.77	36.35 ± 31.79	43.94 ± 39.36	38.66 ± 24.18	37.83 ± 6.32
Veg_87	39.15 ± 3.82	39.78 ± 2.84	45.90 ± 1.54	52.30 ± 1.48	44.28 ± 2.31	33.04 ± 1.74

Table 3 continued

Codes	TSI_{CR1}	TSI_{CR2}	TSI_{CR3}	TSI_{CR4}	TSI_{CR}	TSI_{ZOO}
Veg_17	51.22 ± 1.31	46.27 ± 2.04	43.82 ± 2.39	46.30 ± 1.12	46.90 ± 1.70	45,04 ± 1,54
Vol_84	47.13 ± 4.90	51.92 ± 3.92	60.81 ± 0.84	61,31 ± 1.96	55.29 ± 2.66	45.36 ± 1.17
Vol_85	45.92 ± 6.08	50.55 ± 3.87	60.35 ± 1.21	61.19 ± 2.99	54.50 ± 3.49	46.27 ± 2.23
Vol_86	60.47 ± 2.40	56.24 ± 0.58	59.06 ± 1.02	63.49 ± 3.45	59.81 ± 1.30	57.01 ± 1.14
Vou_16	59.14 ± 15.70	55.35 ± 6.56	58.18 ± 1.41	57.15 ± 2.14	57.45 ± 4.71	53.05 ± 8.86
Yli_90	60.26 ± 2.84	57.06 ± 5.04	58.19 ± 3.69	59.48 ± 4.00	58.75 ± 3.13	55.45 ± 2.62

lakes exhibit prolonged cyanobacterial blooms (Vardaka et al., 2005).

In order to evaluate the application of TSI_{ROT}, TSI_{CR} and TSI_{ZOO} indices in discriminating trophic categories, we used the lakes trophic category based only on phytoplankton biomass since it is a more reliable index compared to TSI_{SD} for Greek lakes (Katsiapi et al., 2016). Thus, the trophic category (oligotrophic, mesotrophic, eutrophic and hypertrophic) of each lake based on mean summer phytoplankton biomass (Table 3) was used and the cases characterized as “meso-eutrophic” and “eu-hypertrophic” were grouped to the mesotrophic and eutrophic category, respectively. ANOVA and Bonferroni correction were applied to reveal if the TSI_{ROT}, TSI_{CR} and TSI_{ZOO} differed among the four groups of trophic state. Weight cases for each parameter were used to reduce bias due to there being different number of lakes or samplings in each group. Furthermore, ANOVA and weight cases were also used to reveal if the TSI_{ROT}, TSI_{CR} and TSI_{ZOO} indices differed among the groups of low (oligotrophic and mesotrophic) and high (eutrophic and hypertrophic) trophic categories.

All statistical analyses were performed using IBM SPSS Statistics 25.

Results

The mean summer values (± standard deviation) of the trophic state indices are given in Table 3, while the values for each sampling are given in Online Resource 1 (Table II). The mean TSI_{SD} ranged from 24.46 ± 2.18 (Lake Trichonis) to 82.65 ± 9.15 (Lake Pamvotis). Mean summer phytoplankton biomass ranged from 0.16 ± 0.05 mg/l (Lake Amvrakia) to 42.24 ± 28.37 mg/l (Lake Voulkaria). The mean TSI_{ROT} ranged from 33.62 ± 2.36 (Lake Tavropos) to 54.06 ± 3.62 (Lake Yliki). In more detail, mean TSI_{ROT1} ranged from 22.82 ± 5.38 (Lake Amvrakia) to 57.07 ± 3.20 (Lake Doirani); mean TSI_{ROT2} ranged from 26.57 ± 3.37 (Lake Vegoritis, 1987) to 63.50 ± 4.49 (Lake Paralimni); mean TSI_{ROT3} ranged from 44.30 ± 0 (Lake Ozeros) to 62.65 ± 1.50 (Lake Yliki) and mean TSI_{ROT4} ranged from 24.72 ± 2.48 (Lake Tavropos) to 51.14 ± 9.56 (Lake Vegoritis, 1987). The mean TSI_{CR} ranged from 32.67 ± 18.77 (Lake Ozeros) to 59.81 ± 1.30 (Lake Volvi, 1986). In more detail, mean TSI_{CR1} ranged from 37.88 ± 0.54 (Lake Tavropos) to 60.47 ± 2.40 (Lake Volvi, 1986); mean TSI_{CR2} ranged from 24.85 ± 21.52 (Lake Ozeros) to 57.06 ± 5.04 (Lake Yliki); mean TSI_{CR3} ranged from 25.29 ± 22.15 (Lake Ozeros) to 60.81 ± 0.80 (Lake Volvi, 1984) and mean TSI_{CR4} ranged from 29.59 ± 25.59 (Lake Ozeros) to 66.18 ± 12.61 (Lake Kastorias, 2016).

The TSI_{ROT} formulae TSI_{ROT1} and TSI_{ROT2} were correlated significantly with both TSI_{SD} [$R^2 = 0.380$, $P < 0.001$ and $R^2 = 0.277$, $P < 0.001$, respectively (Table 4)] (Fig. 2) and phytoplankton biomass [$R^2 = 0.139$, $P = 0.002$ and $R^2 = 0.085$, $P = 0.017$, respectively (Table 4)] (Fig. 3). On the other hand, the formulae TSI_{ROT3} and TSI_{ROT4} were not correlated significantly with either TSI_{SD} [$R^2 = 0.039$, $P = 0.116$ and $R^2 = 0.013$, $P = 0.374$, respectively (Table 4)] (Fig. 2) or phytoplankton biomass [$R^2 = 0.007$, $P = 0.502$ and $R^2 = 0.027$, $P = 0.182$, respectively (Table 4)] (Fig. 3).

The TSI_{CR} formulae TSI_{CR1} and TSI_{CR2} were correlated significantly with both TSI_{SD} [$R^2 = 0.361$, $P < 0.001$ and $R^2 = 0.242$, $P < 0.001$, respectively (Table 4)] (Fig. 2) and phytoplankton biomass

Table 4 Results of linear regression analysis for trophic state indices based on rotifer communities (TSI_{ROT} formulae $TSI_{ROT1} - TSI_{ROT4}$) and crustacean communities (TSI_{CR} formulae $TSI_{CR1} - TSI_{CR4}$) against trophic state index based on transparency (TSI_{SD}) and mean summer phytoplankton biomass (mg/l)

	TSI_{SD}				Phytoplankton biomass			
	Relationship	F	R^2	P	Relationship	F	R^2	P
TSI_{ROT1}	$y = 17.10 + 0.44x$	38.69	0.380	< 0.001	$y = 39.69 + 0.36x$	10.48	0.139	0.002
TSI_{ROT2}	$y = 23.93 + 0.42x$	24.16	0.277	< 0.001	$y = 45.47 + 0.31x$	6.03	0.085	0.017
TSI_{ROT3}	$y = 46.16 + 0.08x$	2.54	0.039	0.116	$y = 50.39 + 0.04x$	0.46	0.007	0.502
TSI_{ROT4}	$y = 35.97 + 0.07x$	0.80	0.013	0.374	$y = 38.69 + 0.13x$	1.82	0.027	0.182
TSI_{CR1}	$y = 30.01 + 0.36x$	35.52	0.361	< 0.001	$y = 48.22 + 0.29x$	10.10	0.134	0.002
TSI_{CR2}	$y = 29.06 + 0.35x$	20.13	0.242	< 0.001	$y = 46.91 + 0.25x$	5.37	0.076	0.024
TSI_{CR3}	$y = 38.84 + 0.29x$	11.34	0.153	0.001	$y = 51.82 + 0.20x$	3.209	0.047	0.078
TSI_{CR4}	$y = 45.33 + 0.19x$	4.05	0.060	0.048	$y = 55.66 + 0.12x$	0.869	0.013	0.355

[$R^2 = 0.134$, $P = 0.002$ and $R^2 = 0.076$, $P = 0.024$, respectively (Table 4)] (Fig. 3). On the other hand, the formulae TSI_{CR3} and TSI_{CR4} were correlated significantly only with the TSI_{SD} [$R^2 = 0.153$, $P = 0.001$ and $R^2 = 0.060$, $P = 0.048$, respectively (Table 4)] (Fig. 2)] and they were not correlated significantly with [or phytoplankton biomass [$R^2 = 0.047$, $P = 0.078$ and $R^2 = 0.013$, $P = 0.355$, respectively (Table 4)] (Fig. 3).

Based on these formulae that significantly correlated with both eutrophication proxies (Figs. 2 and 3, Table 4), we propose a new index, TSI_{ZOO}, as the average of the TSI_{ROT1} (rotifers abundance/numbers), TSI_{ROT2} (rotifers wet biomass), TSI_{CR1} (crustaceans abundance) and TSI_{CR2} (cyclopoids wet biomass). The mean TSI_{ZOO} ranged from 33.04 ± 1.74 (Lake Vegoritis, 1987) to 58.65 ± 0.42 (Lake Doirani) (Table 3).

Linear regressions were also applied to test the dependence of the three zooplanktonic TSI indices, i.e. TSI_{ROT} (the average of $TSI_{ROT1} - TSI_{ROT4}$), TSI_{CR} (the average of $TSI_{CR1} - TSI_{CR4}$) and TSI_{ZOO} (the average of TSI_{ROT1}, TSI_{ROT2}, TSI_{CR1} and TSI_{CR2}), on eutrophication proxies TSI_{SD} and phytoplankton biomass as it is shown in Fig. 4. All indices were significantly correlated ($P < 0.05$) with both eutrophication proxies and the fitted relationships are shown in Table 5. The best-fitted index compared to TSI_{SD} is TSI_{ZOO} ($F = 51.29$, $R^2 = 0.449$) while compared to phytoplankton biomass both TSI_{ROT} and TSI_{ZOO} are almost equally well fitted ($F = 11.86$, $R^2 = 0.154$ and $F = 11.50$, $R^2 = 0.150$, respectively).

The three zooplanktonic TSI indices—TSI_{ROT}, TSI_{CR} and TSI_{ZOO}—were also correlated with the percentage of cyanobacteria to total phytoplankton biomass (Fig. 5). Even though these correlations were not statistical significant ($P > 0.05$), the most influenced index was the TSI_{ROT} ($F = 3.77$, $R^2 = 0.055$, $P = 0.056$) (Table 5).

When the trophic state of each sampling was estimated based on all trophic indices (mean summer phytoplankton biomass, TSI_{SD}, TSI_{ROT}, TSI_{CR} and TSI_{ZOO}), it was observed that they did not identify the same trophic category in all cases (Online Resource 1, Table III). However, when the mean values for the whole summer period were used for each zooplanktonic index (Table 6), using the proposed boundaries by Ejsmont-Karabin (2012) and Ejsmont-Karabin & Karabin (2013) there were some cases identifying the same trophic state [e.g. Trichonis, Vegoritis (1987) and Volvi (1985) (Table 6)]. Still the three zooplanktonic TSI indices (TSI_{ROT}, TSI_{CR} and TSI_{ZOO}) only identified mesotrophic and eutrophic states.

When TSI_{ROT}, TSI_{CR} and TSI_{ZOO} were evaluated based on only the phytoplankton index, they detected different trophic states. Each index was significantly differentiated between the trophic state categories indicated by mean summer phytoplankton biomass (ANOVA, $P < 0.0001$); however, the pairwise test revealed different potentials among the three indices to detect each trophic state. TSI_{ROT} detected different trophic states (ANOVA, $F = 11.628$, $P < 0.0001$) and differences between the oligotrophic lakes and the rest of the trophic

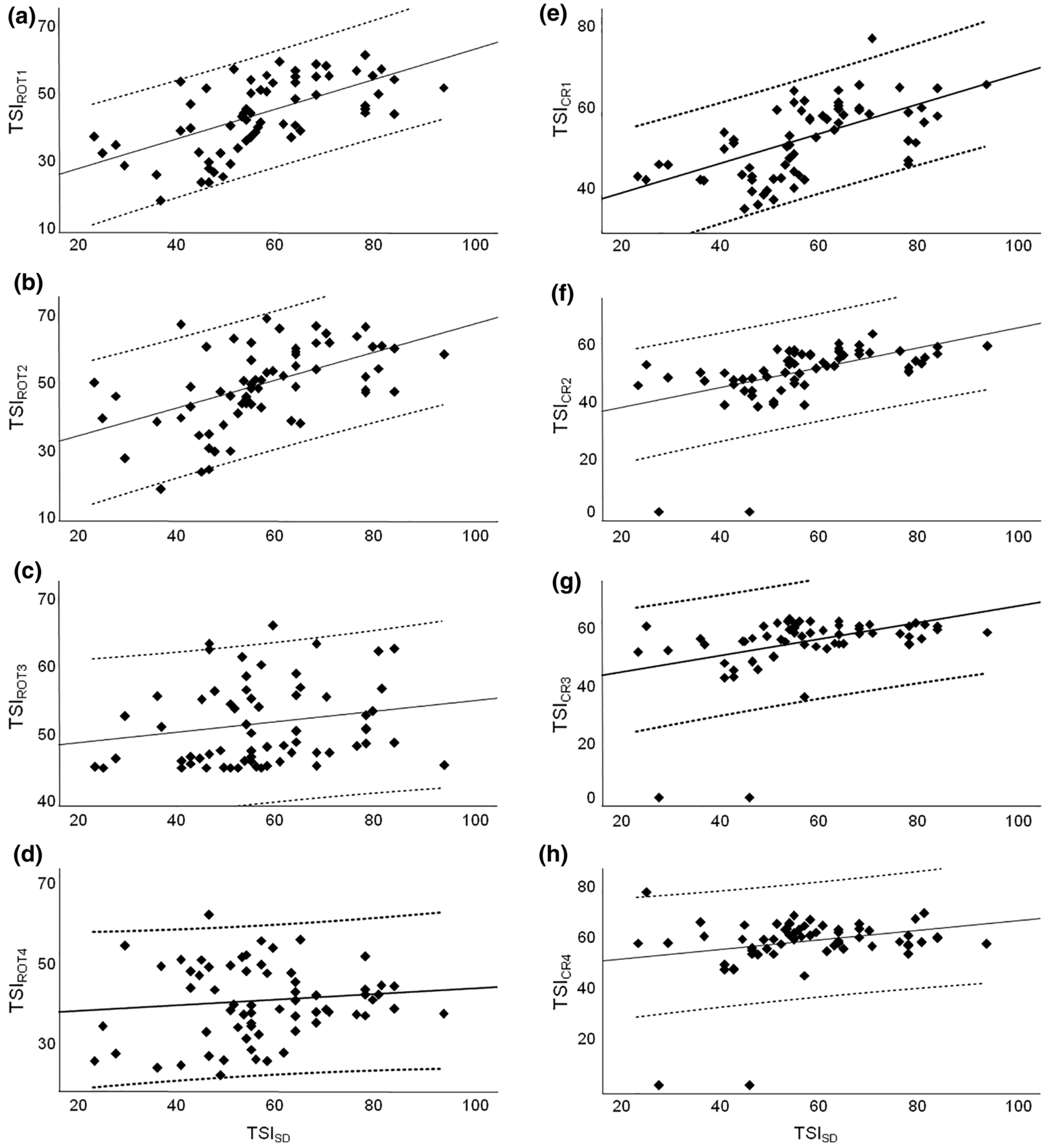

Fig. 2 Scatter plots of TSI$_{ROT}$ formulae **a** TSI$_{ROT1}$, **b** TSI$_{ROT2}$, **c** TSI$_{ROT3}$ and **d** TSI$_{ROT4}$ and TSI$_{CR}$ formulae **e** TSI$_{CR1}$, **f** TSI$_{CR2}$, **g** TSI$_{CR3}$ and **h** TSI$_{CR4}$ against TSI$_{SD}$, the solid line indicates the linear regression line and the dashed lines indicate the (95%) confidence and prediction limits of the model

states were significant (Bonferoni, $P < 0.05$) (Fig. 6). TSI$_{CR}$ detected different trophic states (ANOVA, $F = 7.055$, $P < 0.0001$) discriminating oligotrophic lakes from eutrophic and hypertrophic lakes but not from mesotrophic lakes (Bonferoni, $P < 0.05$) (Fig. 6). TSI$_{ZOO}$ also differentiated

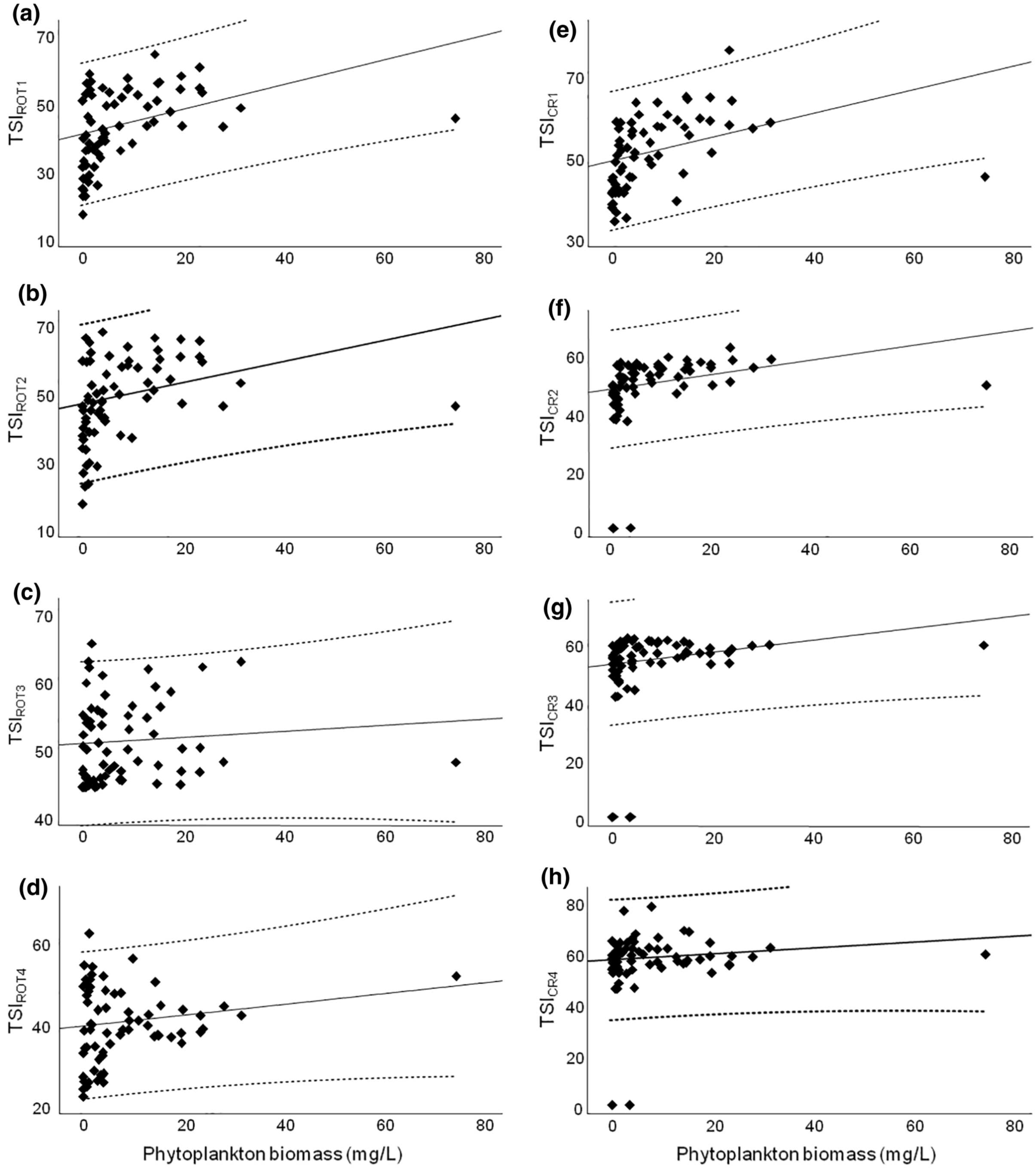

Fig. 3 Scatter plots of $\mathrm{TSI}_{\mathrm{ROT}}$ formulae **a** $\mathrm{TSI}_{\mathrm{ROT1}}$, **b** $\mathrm{TSI}_{\mathrm{ROT2}}$, **c** $\mathrm{TSI}_{\mathrm{ROT3}}$ and **d** $\mathrm{TSI}_{\mathrm{ROT4}}$ and $\mathrm{TSI}_{\mathrm{CR}}$ formulae **e** $\mathrm{TSI}_{\mathrm{CR1}}$, **f** $\mathrm{TSI}_{\mathrm{CR2}}$, **g** $\mathrm{TSI}_{\mathrm{CR3}}$ and **h** $\mathrm{TSI}_{\mathrm{CR4}}$ against phytoplankton biomass (mg/l), the solid lines indicate the linear regression line and the dashed lines indicate the (95%) confidence and prediction limits of the model

significantly between the trophic state categories (ANOVA, $F = 13.688$, $P < 0.0001$) and the pairwise test indicated significant differences between oligotrophic–mesotrophic lakes and eutrophic–hypertrophic lakes (Bonferoni, $P < 0.05$) (Fig. 6).

Based on the Bonferoni tests, we consider that a classification of only two trophic states—the low

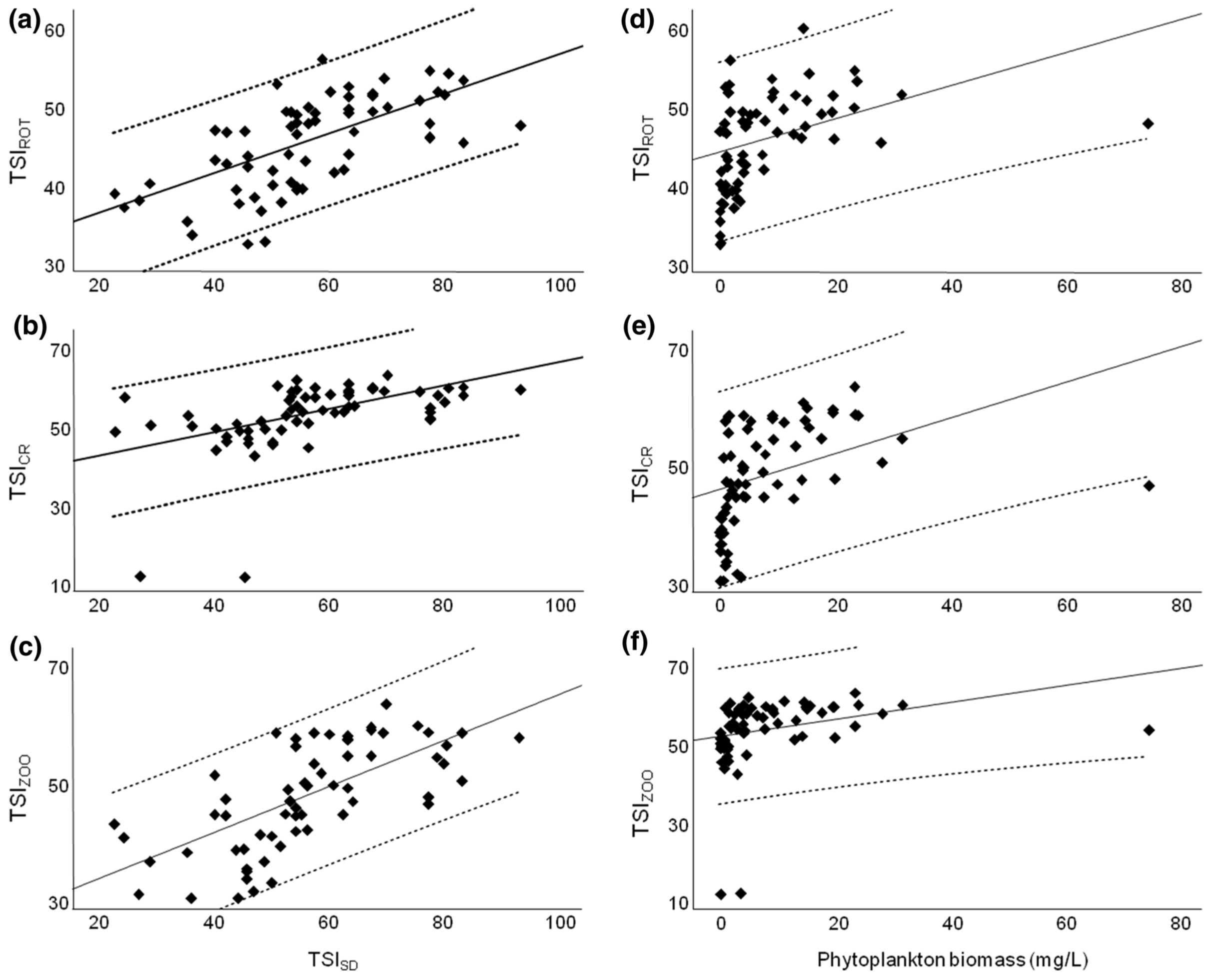

Fig. 4 Scatter plots of TSI_{ROT} (**a**, **d**), TSI_{CR} (**b**, **e**) and TSI_{ZOO} (**c**, **f**) against TSI_{SD} and phytoplankton biomass (mg/l), respectively, the solid lines indicate the linear regression line and the dashed lines indicate the (95%) confidence and prediction limits of the model

(oligotrophic and mesotrophic lakes) and the high (eutrophic and hypertrophic lakes)—might be more appropriate (Fig. 7). Thus, we propose that the boundaries for TSI_{ROT} and TSI_{ZOO} should be set at 45 in order to detect low and high trophic state and for TSI_{CR} the boundary should be set at 50. Using these boundaries, the mean values of the summer period TSI_{ROT} could detect correctly 78% of the lakes trophic state; TSI_{CR} could detect correctly 83% of the lakes trophic state and TSI_{ZOO} could detect correctly 87% of the trophic state according to Table 7. Moreover, all zooplanktonic TSI indices could detect the two trophic categories (low and high) as defined by mean summer phytoplankton biomass (ANOVA TSI_{ROT}: $F = 7.316$, $P = 0.011$, TSI_{CR}: $F = 5.787$, $P = 0.022$ and TSI_{ZOO}: $F = 15.514$, $P < 0.0001$).

Discussion

Rotifer Trophic State Index (TSI_{ROT}) and Crustacean Trophic State Index (TSI_{CR}) are two recently developed indices based on data from zooplankton communities from Polish lakes (Ejsmont-Karabin, 2012; Ejsmont-Karabin & Karabin, 2013) and thus their original forms have been mainly applied in Poland, with TSI_{ROT} being used in more studies (e.g. Gutkowska et al., 2013; Jekatierynczuk-Rudczyk et al., 2014; Dembowska et al., 2015; Marszelewski et al., 2017) as compared to TSI_{CR} (Ochocka & Pasztaleniec, 2016; Dunalska et al., 2018). In different regions, only some of the proposed formulae have been used for TSI_{ROT}; more specifically TSI_{ROT1} has been used in Mexico (Gutiérrez et al., 2017; Moreno-

Table 5 Results of linear regression analysis for trophic state indices based on rotifer communities (TSI_{ROT}), crustacean communities (TSI_{CR}) and zooplankton communities (TSI_{ZOO}) against trophic state index based on transparency (TSI_{SD}), mean summer phytoplankton biomass (mg/l) and the percentage contribution of cyanobacteria to total phytoplankton biomass

	TSI_{SD}				Total phytoplankton biomass (mg/l)				% Cyanobacteria			
	Relationship	*F*	R^2	*P*	Relationship	*F*	R^2	*P*	Relationship	*F*	R^2	*P*
TSI_{ROT}	$y = 30.79 + 0.25x$	43.86	0.410	< 0.001	$y = 43.56 + 0.21x$	11.86	0.154	*0.001*	$y = 42.82 + 0.05x$	3.77	0.055	0.056
TSI_{CRU}	$y = 35.31 + 0.30x$	20.80	0.248	< 0.001	$y = 50.65 + 0.21x$	0.77	0.023	*0.023*	$y = 49.26 + 0.06x$	2.94	0.045	0.091
TSI_{ZOO}	$y = 25.02 + 0.39x$	51.29	0.449	< 0.001	$y = 45.07 + 0.30x$	11.50	0.150	*0.001*	$y = 44.54 + 0.06x$	2.60	0.038	0.112

Gutiérrez et al., 2018) and Nigeria (Bolawa et al., 2018), while Haberman & Haldna (2014) used both TSI_{ROT1} and TSI_{ROT4} in the Estonian Lake Vortsjärv. TSI_{CR} has also been partially used in Nigeria (TSI_{CR5}) (Bolawa et al., 2018) and in Poland (TSI_{CR3}, TSI_{CR4} and TSI_{CR6}) by Jekatierynczuk-Rudczyk et al. (2014). In the Mediterranean zone, only TSI_{ROT1} has been applied in Portugal (Geraldes & Pasupuleti, 2016). The present work is the first application of both TSI_{ROT} and TSI_{CR} for Greek lakes.

We applied TSI_{ROT} and TSI_{CR} as the average of 4 out of the 6 proposed formulae for each index due to data limitations and differences in the trophic state indicator species. Despite the fact that in our attempt to evaluate the TSI_{ROT} index for the Mediterranean lakes, we did not use the formula TSI_{ROT5} (the percentage of *tecta* form in the *K. cochlearis* population) this formula might be suitable for eutrophication assessment, as in the case of the Neva Estuary (Baltic Sea) (Gopko & Telesh, 2013). The same stands for the TSI_{ROT6} and TSI_{CR6} formulae of the indicator species which we did not use because the proposed indicator species do not follow the suggested pattern of dominating only oligotrophic or eutrophic lakes when the Greek lakes were examined. However, they are considered as a suitable tool for trophic state assessment and even as a metric for water quality in Poland (Ochocka & Pasztaleniec, 2016). Still, in order to use these formulae of the indicator species, further research should be done in order for indicator species to be identified for each region in general as well as the Mediterranean. This is further supported by the different patterns of dominance for the same species recorded throughout the literature, e.g. for rotifers the genus *Trichocerca* Lamarck, 1801, is thought to be an indicator of oligotrophic systems by Sládecek (1983); while *Trichocerca capucina* (Wierzejski & Zacharias, 1893) and *Trichocerca pusilla* (Jennings, 1903) are even considered typical of eutrophic conditions (Gulati, 1983); while *D. cucullata* is considered both an indicator of oligotrophic lakes (Karabin, 1985) and typical of eutrophication condition (Pejler, 1983).

In our attempt to find the more efficient formulae for the assessment of Mediterranean systems, TSI_{ROT} and TSI_{CR} formulae were correlated with the eutrophication proxies TSI_{SD} and phytoplankton biomass. Based on the fact that only TSI_{ROT1}, TSI_{ROT2}, TSI_{CR1} and TSI_{CR2} were significantly correlated both with TSI_{SD} and phytoplankton biomass, we propose a new

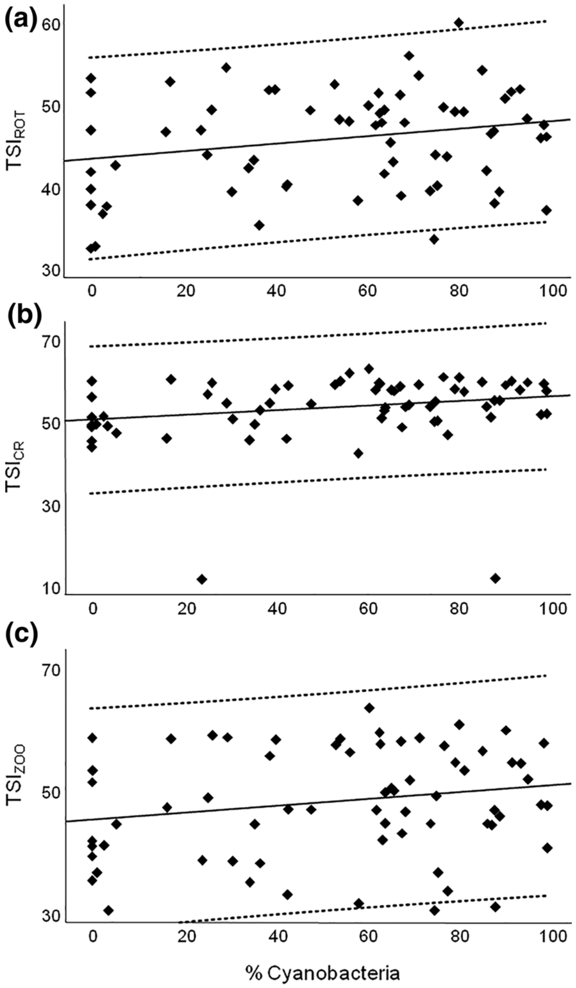

Fig. 5 Scatter plots of **a** TSI$_{ROT}$, **b** TSI$_{CR}$ and **c** TSI$_{ZOO}$ against and the percentage contribution of cyanobacteria to total phytoplankton biomass, the solid lines indicate the linear regression line and the dashed lines indicate the (95%) confidence and prediction limits of the model

index of the whole zooplankton community called TSI$_{ZOO}$. This new index is the average of these formulae, which had the best-fitted model when the three indices used in the present study (TSI$_{ROT}$, TSI$_{CR}$ and TSI$_{ZOO}$) were correlated with the two eutrophication proxies. Hence, TSI$_{ZOO}$ seems to be the most efficient of the three zooplanktonic indices in discriminating eutrophication. Further research could be done in order to relate these indices with other commonly used eutrophication proxies such as nutrients (i.e. total phosphorous) or other TSI indices (i.e. TSI$_{CHL}$).

For the evaluation of the indices used in the present study, we assessed the trophic state of the same lake/sampling using all trophic state indices. The classification of lakes was dissimilar for many cases when all indices or when only the zooplanktonic indices were used. Such discordances among different trophic indices have been recorded both in reservoirs and natural lakes (e.g. Duggan et al., 2001; García-Chicote et al., 2018). Differences between the trophic indices were smoothened when the mean values of the summer period were used, e.g. Lake Volvi in 1985 was characterised as mesotrophic up to eutrophic using monthly values of all three zooplanktonic TSI indices while only as meso-eutrophic when mean values were used, thus, we propose the trophic state estimation to be done based on the mean values of the zooplanktonic indices.

So far all studies that have applied the TSI$_{ROT}$ and TSI$_{CR}$ indices in order to evaluate them only compared their values with the trophic state as indicated by TSI indices or even by other indices based on zooplankton (e.g. Jekatierynczuk-Rudczyk et al., 2014; Gutiérrez et al., 2017), except Dembowska et al. (2015) who correlated TSI$_{ROT}$ with phytoplankton indices. In the present study, acknowledging that phytoplankton is the most reliable element for assessing trophic state (e.g. Katsiapi et al., 2016), we evaluated all indices based on the trophic state as indicated by mean summer phytoplankton biomass. We found that TSI$_{SD}$ overestimated the trophic state of Lake Ozeros due to increased detritus as well as the reservoirs (Tavropos and Kremasta), since riverine systems have increased organic material (total suspended solids or allochthonous-dissolved organic matter) lowering transparency (e.g. Mash et al., 2004; Bolgrien et al., 2009). TSI$_{SD}$ also underestimated the trophic state of the deep lakes Trichonis and Vegoritis. Despite these differentiations, Katsiapi et al. (2016) propose the use of TSI$_{SD}$ for practical large-scale and long-term monitoring purposes of Mediterranean lakes when combined with low-frequency high-quality phytoplankton data during the warm period (June to October). However, it should not be used for the calibration of other eutrophication indices, thus the rest of our analyses were made using only the phytoplankton biomass as a eutrophication proxy.

Regarding the zooplanktonic TSI indices and their proposed limits (Ejsmont-Karabin, 2012; Ejsmont-Karabin & Karabin, 2013), oligotrophic lakes were overestimated since all lakes with TSI < 45 were grouped as mesotrophic and hypertrophic lakes were

Table 6 The trophic categories (Oligo: oligotrophic, Meso: mesotrophic, Eu: eutrophic, Hyper: hypertrophic) identified for each lake using the mean values of different trophic state indices [TSI_{SD}, summer phytoplankton biomass (PB), TSI_{ROT}, TSI_{CR} and TSI_{ZOO}]

Codes	TSI_{SD}	PB	TSI_{ROT}	TSI_{CR}	TSI_{ZOO}
Amv_16	Oligo	Oligo[a]	Meso	Meso–Eu	Meso
Doi_04	Hyper	Hyper	Meso–Eu	Eu	Eu
Kas_99	Hyper	Eu–Hyper	Meso–Eu	Eu	Eu
Kas_16	m.d.	Eu	Meso–Eu	Eu	Eu
Kre_16	Eu	Oligo	Meso	Meso–Eu	Meso
MgP_16	Meso	Meso–Eu	Meso	Meso–Eu	Meso
MkP_90	Eu	Hyper	Meso–Eu	Eu	Meso–Eu
MkP_91	Eu	Eu	Meso–Eu	Eu	Meso–Eu
MkP_92	Eu	Eu	Meso	Meso–Eu	Meso–Eu
MkP_16	Eu	Meso	Meso–Eu	Meso–Eu	Meso
Oze_16	Meso	Oligo[b]	Meso–Eu	Meso	Meso–Eu
Pam_16	Hyper	Hyper	Meso–Eu	Meso–Eu	Meso–Eu
Par_17	Eu	Meso	Meso–Eu	Eu	Eu
Pet_10	Eu	Eu	Meso-Eu	Eu	Eu
Tav_87	Meso	Oligo	Meso	Meso–Eu	Meso
Tri_16	Oligo	Eu	Meso	Meso	Meso
Veg_87	Meso	Meso–Eu	Meso	Meso	Meso
Veg_17	Meso	Eu	Meso	Meso–Eu	Meso–Eu
Vol_84	Eu	Eu	Meso	Eu	Meso–Eu
Vol_85	Eu	Eu	Meso–Eu	Meso-Eu	Meso–Eu
Vol_86	Eu	Eu	Meso–Eu	Eu	Eu
Vou_16	Hyper	Hyper	Meso–Eu	Eu	Meso–Eu
Yli_90	Hyper	Hyper	Meso–Eu	Eu	Eu

Codes abbreviations according to Table 3, m.d.: missing data

[a] Lake Amvrakia can be allocated as oligotrophic based on biomass but having the information of the total dominance of cyanobacteria and specifically of *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek, 1988, we should investigate the biomass distribution deeper than the euphotic zone

[b] Lake Ozeros can be allocated as oligotrophic based on biomass but the dominance of detritus could indicate the need for more investigation on the trophic state

underestimated since none of the three indices took values over 65. TSI_{ROT} underestimated also the majority of the studied eutrophic lakes. TSI_{ROT} had lower values compared to TSI_{CR} leading in estimation of different categories for many cases. This was expected due to the increased impact of fish predation on crustacean (Ejsmont-Karabin & Karabin, 2013). However, TSI_{CR} had very low values in the samplings of July of lakes Trichonis and Ozeros because cyclopoid copepods were not recorded in crustacean's abundance resulting in zero values for the TSI_{CR2}, TSI_{CR3} and TSI_{CR4} formulae, indicating that TSI_{CR} should not be used in lakes without cyclopoids or when no cyclopoids are recorded. TSI_{ZOO} being the average of the two indices had better results estimating the trophic state, strengthening the result of being the index most depended on eutrophication.

Estimations of different trophic states using different indices, especially for TSI_{ROT}, have been recorded elsewhere as well, e.g. in Madín Reservoir in Mexico (Moreno-Gutiérrez et al., 2018). Even in Polish lakes in some cases TSI_{ROT} underestimates the trophic state for eutrophic and hypertrophic lakes (Jekatierynczuk-Rudczyk et al., 2014). Cyanobacterial blooms have been identified as one possible reason for these misclassifications when TSI_{ROT} is applied

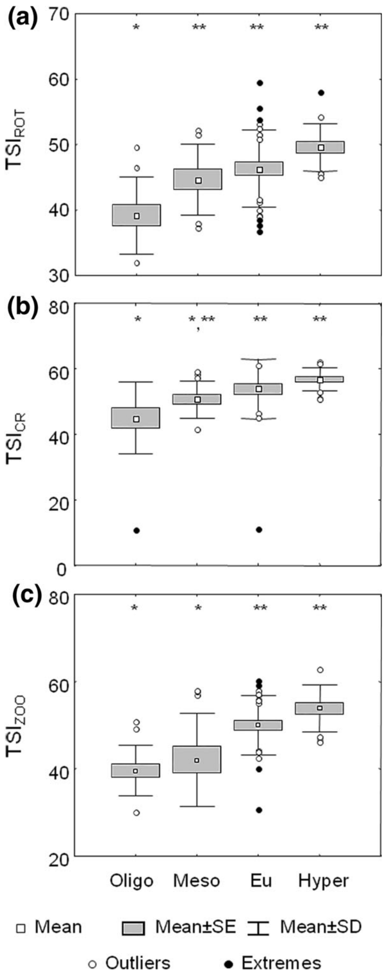

Fig. 6 Box plots of **a** TSI_{ROT}, **b** TSI_{CR} and **c** TSI_{ZOO} based on data of zooplankton communities from the 16 Greek lakes grouped by trophic state (indicated by mean summer phytoplankton biomass). *, **, *** Significant differences (Bonferoni procedure)

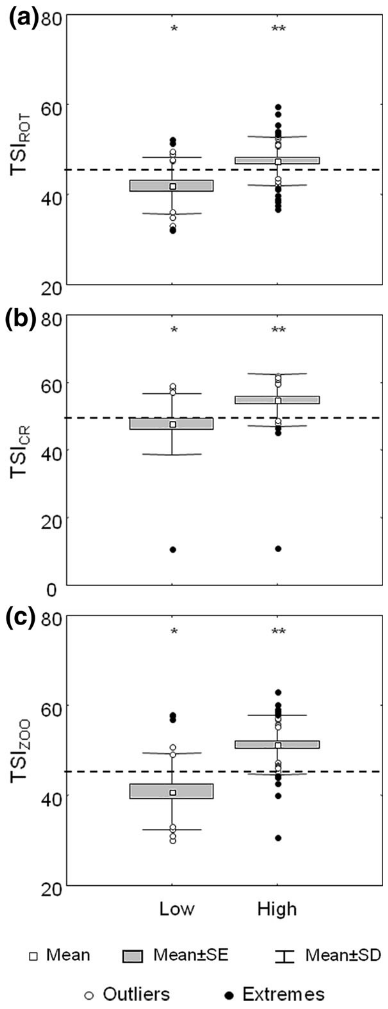

Fig. 7 Box plots of **a** TSI_{ROT}, **b** TSI_{CR} and **c** TSI_{ZOO} based on data of zooplankton communities from the 16 Greek lakes grouped into low (oligotrophic–mesotrophic) and high (eutrophic–hypertrophic) trophic groups (indicated by mean summer phytoplankton biomass). The dashed line indicates the boundary proposed in the present study for each index. *, ** Significant differences (Bonferoni procedure)

(Dembowska et al., 2015). Mediterranean freshwater systems can exhibit prolonged cyanobacterial blooms (up to 8 months) compared to temperate lakes, and their phytoplankton community can even be dominated by cyanobacteria up to more than 90% in eutrophic lakes (Vardaka et al., 2005). This was also the case in the present study, where cyanobacteria contributed to the total biomass in all lakes, except Kremasta Reservoir, and were the dominant group in terms of biomass in lakes of the entire trophic spectrum [i.e. their contribution reached up to 75.98% in the oligotrophic lake Amvrakia cyanobacteria, 99.96% in the deep lake Trichonis and up to 100% in the hypertrophic lake Pamvotis (Online Resource 1, Table II)]. Nevertheless, despite the increased cyanobacteria contribution, no statistically significant relationships with the zooplanktonic TSI indices used in the present study were recorded.

Despite discrepancies in trophic state estimations, all zooplanktonic indices (TSI_{ROT}, TSI_{CR}, TSI_{ZOO}) were significantly differentiated among the groups of trophic state indicated by mean summer phytoplankton biomass. It is of interest that TSI_{ROT} differentiated significantly only for the oligotrophic state. Even though the dataset used for TSI_{ROT} development

Table 7 Mean TSI_{SD} and mean summer phytoplankton biomass (PB) identified the trophic categories (Oligo: oligotrophic, Meso: mesotrophic, Eu: eutrophic, Hyper: hypertrophic) for each lake and mean TSI_{ROT}, TSI_{CR} and TSI_{ZOO} identified the low and high trophic categories using the boundaries proposed in the present study

Codes	TSI_{SD}	PB	TSI_{ROT}	TSI_{CR}	TSI_{ZOO}
Amv_16	Oligo	Oligo[a]	Low	High	Low
Doi_04	Hyper	Hyper	High	High	High
Kas_99	Hyper	Eu–Hyper	High	High	High
Kas_16	m.d.	Eu	High	High	High
Kre_16	Eu	Oligo	Low	Low	Low
MgP_16	Meso	Meso–Eu	Low	High	Low
MkP_90	Eu	Hyper	High	High	High
MkP_91	Eu	Eu	High	High	High
MkP_92	Eu	Eu	Low	High	High
MkP_16	Eu	Meso	High	Low	Low
Oze_16	Meso	Oligo[b]	High	Low	High
Pam_16	Hyper	Hyper	High	High	High
Par_17	Eu	Meso	High	High	High
Pet_10	Eu	Eu	High	High	High
Tav_87	Meso	Oligo	Low	Low	Low
Tri_16	Oligo	Eu	Low	Low	Low
Veg_87	Meso	Meso–Eu	Low	Low	Low
Veg_17	Meso	Eu	Low	Low	High
Vol_84	Eu	Eu	Low	High	High
Vol_85	Eu	Eu	High	High	High
Vol_86	Eu	Eu	High	High	High
Vou_16	Hyper	Hyper	High	High	High
Yli_90	Hyper	Hyper	High	High	High

Codes abbreviations according to Table 3, *m.d.* missing data

[a] Lake Amvrakia can be allocated as oligotrophic based on biomass but having the information of the total dominance of cyanobacteria and specifically of *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek, 1988, we should investigate the biomass distribution deeper than the euphotic zone

[b] Lake Ozeros can be allocated as oligotrophic based on biomass but the dominance of detritus could indicate the need for more investigation on the trophic state

included lakes covering the mesotrophic to the hypertrophic trophic spectrum (Ejsmont-Karabin, 2012), oligotrophic lakes might be differentiated from mesotrophic even in temperate lakes. However, further research is needed based on a bigger dataset of temperate lakes in order to clarify this, and to propose, if possible, a boundary between oligotrophic and mesotrophic lakes. TSI_{CR} on the other hand differentiated significantly the oligotrophic category from the eutrophic and hypertrophic categories and TSI_{ZOO} differentiated oligotrophic and mesotrophic lakes from eutrophic and hypertrophic lakes. Based on the above, there is a need to modify the boundaries proposed by Ejsmont-Karabin (2012) and Ejsmont-Karabin & Karabin (2013); however, value overlaps do not show a clear pattern. Based on the fact that there is no clear differentiation among the trophic categories, we propose that zooplanktonic indices should be used in order to differentiate two trophic groups: one of the low trophic state that includes oligotrophic and mesotrophic lakes, and one of high trophic state that includes eutrophic and hypertrophic lakes. When this categorisation was used also for the trophic state defined by the phytoplankton biomass, all three zooplanktonic indices (TSI_{ROT}, TSI_{CR}, TSI_{ZOO}) significantly differentiated the two categories. The boundaries for discriminating the categories of low and high trophic state for TSI_{ROT} and TSI_{ZOO} could remain TSI = 45 as already proposed by Ejsmont-Karabin (2012) ($TSI < 45$ mesotrophic or low trophic state) but for TSI_{CR}, we propose the boundary to be $TSI_{CR} < 50$, a bit higher than the proposed boundary by Ejsmont-Karabin & Karabin (2013). When the above boundaries were used, TSI_{ZOO} classified correctly the majority of the studied lakes, except Lake Trichonis which was underestimated possibly due to the increased fish predation by *Atherina boyeri* Risso, 1810 (Chrisafi et al., 2007) and the shallow lakes Ozeros and Paralimni which were overestimated; Lake Ozeros had increased abundance of detritus indicating a possible higher trophic state and Lake Paralimni had increased rotifers abundance and domination (> 70%).

In conclusion, we have shown that the Rotifer Trophic State Index (TSI_{ROT}) and the Crustacean Trophic State Index (TSI_{CR}) applied to the zooplankton communities from 16 Greek lakes are increasing across eutrophication in Mediterranean lakes. Furthermore, a new index TSI_{ZOO}, the Zooplankton Trophic State Index, was proposed as the average of the formulae which were significantly correlated with the eutrophication proxies (TSI_{SD} and phytoplankton biomass). The evaluation of the indices indicated that they can detect efficiently two groups of low (oligotrophic–mesotrophic) trophic and high (eutrophic–hypertrophic) trophic state in the Mediterranean region. Moreover, despite the range overlaps, we propose the boundaries to be < 45 for the low category for TSI_{ROT} and TSI_{ZOO} and < 50 for TSI_{CR} when applied in the Mediterranean region. TSI_{ROT} and TSI_{CR} can be promising and useful tools for trophic state estimation because they do not require species-level identifications and are relatively easy and inexpensive to measure; however, TSI_{CR} should not be used in lakes without cyclopoid copepods. TSI_{ZOO}, which represents the entire zooplankton community, was better related to eutrophication and had better estimations of the trophic state especially when the mean summer value was used. Thus, we propose TSI_{ZOO} as a promising and effective tool for monitoring and assessment of eutrophication of Mediterranean lakes since it can be an easy and cost-effective tool in large-scale and long-term monitoring programmes and it could be considered as a parameter of a multimetric index of ecological water quality, based on zooplankton.

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