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The neglected zooplankton communities as indicators of ecological water quality of Mediterranean lakes

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ABSTRACT

The neglected zooplankton communities as indicators of ecological water quality of Mediterranean lakes

The exclusion of zooplankton as a biological quality element from the Water Framework Directive, led to the decreased monitoring of zooplankton communities all over Europe, including Greece. Patterns of abundance, biomass and size structure were investigated in 13 natural Greek lakes covering the whole trophic spectrum and representing various aspects of ecological water quality (high, good, moderate, poor), to identify metrics representing zooplankton community structure and size for ecological water quality assessment in the Mediterranean area. In the studied lakes, rotifers had generally the highest contribution in zooplankton abundance, while copepods had the highest contribution in zooplankton biomass. Small-bodied zooplankton probably dominated due to intense fish predation and prolonged cyanobacterial blooms. Based on the variation of abundance, biomass and the size structure five zooplankton metrics, i.e. total abundance (A_{Zoo}), total dry biomass (B_{Zoo}), zooplankton ratio (R_{Zoo}), mean zooplankton weight (MW_{Zoo}) and cladocerans ratio (R_{Clad}), were tested against phytoplankton biomass as the eutrophication state variable and PhyCoI as the phytoplankton ecological water quality index. All five zooplankton metrics were significantly correlated with phytoplankton biomass and/or identified different trophic state classes and were also correlated with ecological water quality. The five studied zooplankton metrics were found to be promising indicators for ecological water quality assessment and as such we propose them to be included in the monitoring programs of natural Mediterranean lakes.

Key words: ecological water quality, Greek lakes, zooplankton metrics, zooplankton ratios

RESUMEN

Las comunidades olvidadas de zooplancton como indicadoras de la calidad ecológica de lagos mediterráneos

La exclusión del zooplancton como elemento de calidad biológica en la Directiva Marco del Agua ha implicado la reducción en el esfuerzo de monitoreo de las comunidades de zooplancton a lo largo de toda Europa, incluida Grecia. Se investigaron los patrones de abundancia, biomasa y estructura de tamaños en 13 lagos griegos, que incluyen todo el espectro trófico y diversos niveles de calidad (alta, buena, moderada y pobre), para identificar variables relativas a la estructura y tamaño de la comunidad de zooplancton útiles para evaluar la calidad de las masas de agua en el área mediterránea. En los lagos estudiados, los rotíferos fueron los organismos que mostraron mayores abundancias, mientras que los copépodos fueron los que contribuyeron más a la biomasa total de zooplancton. El zooplancton de menor tamaño predominó debido, probablemente, a la intensa depredación ejercida por los peces y a las frecuentes proliferaciones masivas de cianobacterias. A partir de la variación en la abundancia, la biomasa y la estructura de tamaños de la comunidad de zooplancton se compararon cinco variables métricas de zooplancton; abundancia total (A_{Zoo}), biomasa seca total (B_{Zoo}), proporción de zooplancton (R_{Zoo}), peso medio de zooplancton (MW_{Zoo}) y proporción de cladóceros (R_{Clad}); con la biomasa de fitoplancton, utilizada como variable del estado trófico, y PhyCoI como índice fitoplanctónico de calidad ecológica. las cinco métricas de zooplancton se correlacionaron significativamente con la biomasa de fitoplancton, y/o con el estado trófico, así como con la calidad ecológi-

ca de la masa de agua. Las cinco métricas basadas en zooplancton estudiadas fueron indicadores útiles en la evaluación de la calidad ecológica, y proponemos que sean incluidos en los programas de monitoreo de los lagos mediterráneos.

Palabras clave: *calidad ecológica de masas de agua, lagos griegos, métricas de zooplancton, proporciones de zooplancton*

INTRODUCTION

Over the last two decades, the assessment of ecological water quality of freshwater bodies based on biological indices constitutes a main field of research worldwide due to the development of different legislation acts [e.g. the Water Framework Directive (WFD) in Europe and the US Clean Water Act (CWA)]. In Europe, the Water Framework Directive 2000/60/EC has as a core feature the classification of the ecological status of water bodies based on biological quality elements (BQEs), i.e. phytoplankton, benthic macroinvertebrates, fish, aquatic macrophytes and phytobenthos (European Commission, 2000), excluding zooplankton, though without a well-argued scientific explanation for its omission (e.g. Moss, 2007, Caroni & Irvine, 2010, Jeppesen *et al.*, 2011).

Zooplankton has a pivotal role in the pelagic food web of lakes as an intermediate link between phytoplankton and fish mediating energy flow, thus it responds immediately to changes resulting from trophic cascades either through bottom-up or top-down control (Carpenter *et al.*, 1985; Sommer, 2008; Špoljar *et al.*, 2018). The value of zooplankton as an indicator of ecological/environmental conditions is undeniable (e.g. Caroni & Irvine, 2010, Jeppesen *et al.*, 2011); it has been used for ecological water quality assessment (e.g. Kane *et al.*, 2009; Haberman & Haldna, 2014), trophic state assessment (e.g. Ejsmont-Karabin & Karabin, 2013; Špoljar, 2013; Tasevska *et al.*, 2017), and for detecting anthropogenic pressures (Stamou *et al.*, 2017; Kuczyńska-Kippen, 2020).

It has been efficiently used in monitoring programs of lakes tracking changes in the abundance and biomass of the main zooplankton groups, responding to short term changes such as sudden fish kills or lake restoration attempts (Gannon & Stemberger, 1978; Jeppesen *et al.*, 2011; Haberman & Haldna, 2014). However, after the implementation of the WFD in the European Union, national authorities reduced and even excluded zooplankton studies from monitoring programs all over Europe (Jeppesen *et al.*, 2011) creating a gap of knowledge related to the function of the plankton food web. This is even more important for not well-studied ecosystems such as the Mediterranean ones. Patterns described based on the knowledge derived from the well-studied cold-temperate European lakes differentiate in other climatic zones, as the Plankton Ecology Group model (PEG-model) does for polar, tropical (De Senerpont Domis *et al.*, 2013) and Mediterranean lakes (Moustaka-Gouni *et al.*, 2014). Mediterranean lakes are differentiated from temperate ones through morphometric (e.g. larger basin size/lake size), and hydrological (strong seasonality in water supply) characteristics and climate such as the increased availability of solar radiation in winter months (Alvarez Cobelas, 2005; Moustaka-Gouni *et al.*, 2014). As a result, these systems have also biologically important features: a) no biological winter (continuous increase of phytoplankton mainly during late-autumn and winter months) and b) strong fish predation leading to small-bodied zooplankton dominance and thus lower grazing pressure on phytoplankton (Moustaka-Gouni *et al.*, 2014). Acknowledging the particularities of the Mediterranean climatic zone, various indices have been developed lately for the assessment of the ecological water quality of Mediterranean lakes using different BQEs (e.g. Quintana *et al.*, 2015; Katsiapi *et al.*, 2016a; Petriki *et al.*, 2017).

Zooplankton was initially included in two metrics [ratios: dry biomass ratio of crustacean to phytoplankton and cladocerans ratio (R_{Clad})] of the ECOFRAME scheme, a pan-European typology and ecological classification system developed for the water quality assessment of shallow lakes (Moss *et al.*, 2003). Later, Caroni & Irvine (2010) and Jeppesen *et al.* (2011) while calling attention to the omission of zooplankton from the WFD, point out promising zooplankton metrics [e.g. mean weight of different groups, cladoceran fossils] for assessing ecological lake water quality in Europe. Lately, zooplankton research has focused both on the development of zooplankton indices and the finding of appropriate metrics. So far, two zooplankton indices Zooplankton Reservoir Trophic Index (ZRTI) (Montagud *et al.*, 2019) and Zooplankton Biotic Index (ZBI) (De-Carli *et al.*, 2019) have been developed for lakes ecological water quality assessment; both being case specific, requiring the calculation of optimal/tolerant values for the species of each studied region according to their correlation with environmental data (De-Carli *et al.*, 2019). As for quantitative and easy to use zooplankton metrics, they were tested for the assessment of the trophic state (Montagud *et al.*, 2019; Stamou *et al.*, 2019a) and ecological water quality assessment of Mediterranean lakes [e.g, Grazing Potential index (GP) was used as a supportive factor to phytoplankton ($PhyCoI_{GP}$ index) (Stamou *et al.*, 2019b); abundance was proposed for the monitoring of reservoirs (García-Chicote *et al.*, 2018)]. Furthermore, community size structure (zooplankton size in general and especially cladoceran size) can indicate 'top-down' processes, while zooplankton biomass, the proportion of rotifers and calanoid copepods can be indicators of 'bottom-up' processes (Jeppesen *et al.*, 2011) and they are also being tested for water quality assessment of natural lakes, reservoirs and coastal areas in Europe (e.g. Jeppesen *et al.*, 2011; Haberman

Table 1. Information about lakes' characteristic [topographic (Lat: latitude and Long: longitude) and morphological data), sampling years, the respective literature of the zooplankton and phytoplankton data, mean values and standard deviation of total phytoplankton biovolume (PB) and PhyCoI index, trophic state, and ecological water quality for the sampling period of the 13 studied Greek lakes. Lake abbreviations based on figure 1 (n.d.: no data available). *Características de los lagos (topográfica (Lat: latitud y Largo: longitud) y datos morfológicos), años muestreados, referencias bibliográficas de los datos de zooplancton y fitoplancton (respectivamente), valores medios y desviación estándar del biovolumen total de fitoplancton (PB) e índice PhyCoI, estado trófico, y calidad ecológica en los 13 lagos griegos para el periodo de estudio. Las abreviaturas están basadas en la figura 1 (n.d.: datos no disponibles).*

Lake.	Lat	Long	Surface area (Km^2)	Altitude (m a.s.l.)	Max depth (m)	Sampling Year	Studies[1]	PB[3] (mg/L)	Trophic state[3]	PhyCoI[4]	Ecological Water Quality[4]
Amv	38°45'	21°11'	14.5	25	53	2016	a	0.16 ± 0.05	Oligo	4.27 ± 0.23	High
Doi	41°13'	22°46'	34.8	142	8	2004	a	21.04 ± 2.23	Hyper	1.73 ± 0.15	Poor
Kas	40°32'	21°17'	30	629	9	1999	b	13.39 ± 3.48	Eu	n.d.	n.d.
						2016	a	8.04 ± 6.52	Eu	2.40 ± 0.89	Moderate
Lys	38°34'	21°22'	13	14.5	9	2016	c	0.38 ± 2.66	Meso	3.13 ± 0.89	Good
MkP	40°46'	21°05'	39.2	850	9	1990	d	19.87 ± 11.04	Hyper	1.77 ± 0.38	Poor
						1991	d	4.08 ± 2.25	Eu	2.63 ± 0.49	Moderate
						1992	d	5.32 ± 2.07	Eu	2.47 ± 0.29	Moderate
						2016	e	1.20 ± 0.54	Meso	3.97 ± 0.06	Good
Pam	39°39'	20°53'	22	470	9.2	2016	a	16.44 ± 3.14	Hyper	1.53 ± 0.21	Poor
Par	38°27'	23°20'	10	51	8	2017	a	2.20 ± 1.69	Meso	3.40 ± 0.26	Good
Pet	40°44	21°41'	11	572	6	2010	f	9.83 ± 1.17	Eu	2.20 ± 0	Moderate
Tri	38°34'	21°35'	97.2	16	59	2016	a	2.45 ± 1.22	Eu	3.30 ± 0.53	Good
Veg	40°45'	21°47'	46	524	52	1987	g	1.90 ± 0.98	Meso-Eu	n.d.	n.d.
						2017	a	2.39 ± 1.89	Eu	3.50 ± 0.52	Good
Vol	40°40'	23°31'	68.6	37	28	1984	h	2.76 ± 0.51	Eu	2.67 ± 0.12	Moderate
						1985	h	8.33 ± 4.27	Eu	1.80 ± 0.35	Poor
						1984	h	4.00 ± 2.01	Eu	2.70 ± 040	Moderate
Vou	38°52'	20°50'	9.4	5	2.5	2016	a	42.24 ± 28.37	Hyper	1.37 ± 0.23	Poor
Yli	38°24'	23°16'	23.0	78	34	1990	g	18.66 ± 7.68	Hyper	n.d.	n.d.

1 References for data of zooplankton communities: a) Stamou *et al.* (2019a), b) Moustaka-Gouni *et al.* (2006), c) Stamou *et al.* (2019b), d) Michaloudi *et al.* (1997), e) Katsiapi *et al.* (2016b), f) Vourka (2011), g) Moustaka-Gouni *et al.* (2014) and h) Zarfdjian *et al.* (1990)

3 Lakes' trophic state (oligo: Oligotrophic, meso: Mesotrophic, eu: Eutrophic and hyper: Hypertrophic) based on the mean summer phytoplankton biovolume according to Stamou *et al.* (2019a) except for Lake Lysimachia according to Stamou *et al.* (2019b)

4 Lakes' ecological water quality based on PhyCoI index according to Stamou *et al.* (2019b)

& Haldna, 2014; Gorokhova *et al.*, 2016).

The aim of our study was to further examine how zooplankton metrics (used or proposed for temperate lakes) can contribute to the ecological water quality assessment of natural Mediterranean lakes during the warm period, the biomonitoring period followed during the implementation of WFD. For that, we initially examined the summer zooplankton communities of 13 natural Greek lakes along the entire trophic spectrum and of a wide range of morphometry. The dataset derived from published data of various years from 1984 to 2017. We hypothesized that zooplankton metrics based on the variability of abundance, biomass and the body size of the main groups during the warm period could contribute to a screening classification of ecological water quality of Mediterranean lakes. In order to test this hypothesis, five zooplankton metrics were correlated with phytoplankton biomass, a state variable of eutrophication, and with the PhyCoI index, a phytoplankton-based ecological water quality index. Moreover, we examined the ability of zooplankton metrics in distinguishing trophic state and/or ecological water quality classes.

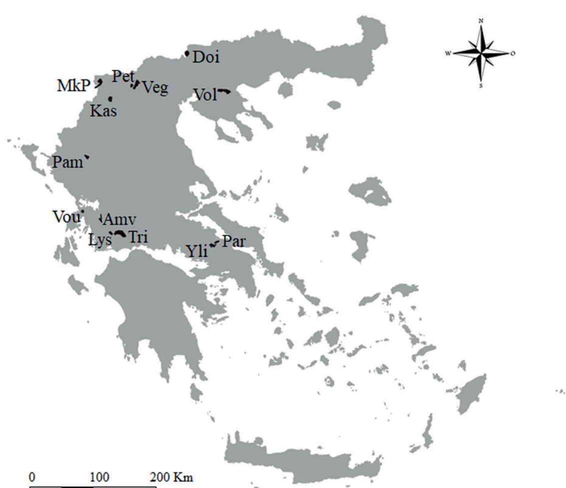

Figure 1. Map of Greece showing the locations of the 13 lakes included in the study. *Mapa de Grecia con la ubicación de los 13 lagos incluidos en el estudio.* Abbreviations: Amvrakia (Amv), Doirani (Doi), Kastoria (Kas), Lysimachia (Lys), Mikri Prespa (MkP), Pamvotida (Pam), Paralimni (Par), Petron (Pet), Trichonis (Tri), Vegoritis (Veg), Volvi (Vol), Voulkaria (Vou), Yliki (Yli).

MATERIALS AND METHODS

Data collection and analysis

The present study comprised data from 13 natural Greek lakes (Fig. 1), which encompass a various range of topographic (altitude) and morphological characteristics (i.e. surface area, mean and maximum depth), and cover the entire trophic state spectrum (Table 1). The present dataset comprised published data from 1984 to 2017 (Table 1) and the number of sampling years per lake ranged from 1 to 4 (3 samplings were conducted during the warm period June to September during each year). In cases with more than one sampling years, the different sampling years were treated like separate lakes in our statistical analyses. Thus, a total number of 20 summer seasons (sampling years) were used. This sampling period is the common period proposed by the WFD for ecological water quality assessment of lakes for all BQE's (European Commission, 2003). Zooplankton sampling was performed following the same sampling protocol described by Mazaris *et al.* (2010) for all lakes. Integrated water samples from the whole water column were collected using a 2-L Niskin sampler at the deepest area of each lake and filtered through a filter of mesh size 50 μm. At least 30 L of water were filtered each time and the samples were preserved in 4 % formalin. Detailed information regarding the microscopic analyses of zooplankton identification, abundance and dry biomass calculation are described in Stamou *et al.* (2019a). Phytoplankton data for the same period are available (Table 1). For the presentation of zooplankton community patterns, the mean value of the 3 samplings during the warm period was used for each sampling year.

Zooplankton metrics

Zooplankton metrics were applied based on the abundance and biomass of the three main groups of zooplankton (Rotifera, Cladocera and Copepoda), i.e. total abundance (A_{Zoo}), total dry biomass (B_{Zoo}), zooplankton ratio (R_{Zoo}), mean zooplankton weight (MW_{Zoo}) and cladocerans ratio (R_{Clad}). These metrics have various responses to degradation (Table 2) and have been used in indices of water quality assessment [ECOFRAME scheme (Moss *et al.*, 2003) and P-IBI (Planktonic Index of Biotic Integrity) (Kane *et al.*, 2009)] and/or have been proposed as promising indicators for ecological water quality assessment in Europe (Caroni & Irvine, 2010; Jeppesen *et al.*, 2011).

Statistical analysis

In order to test if the five zooplankton metrics are efficient for ecological water quality assessment in the Mediterranean region, the metrics were tested against both ecological water quality and eutrophication. Ecological status is an expression of the quality of the structure and functioning of aquatic ecosystems" [paragraph 21 of Article 2, European Commission (2000)]. Thus, for the test against water quality, PhyCoI index was used as a functional innovative phytoplankton-based index developed based on data of Greek lakes [PhyCoI is an ecologically sound index covering all the individual properties of phytoplankton community (i.e. diversity, composition, dominance, biomass, blooms) and can effectively assess the status and ecological integrity of Mediterranean lakes (Katsiapi *et al.*, 2016a; Stamou *et al.*, 2019b)]. Moreover, according to Hering *et al.* (2006) an effective multimetric index and its metrics should be correlated with eutrophication, since it is one of the main features of lake degradation, to enhance its reliability and robustness. Hence, the metrics were also tested against phytoplankton biovolume an accurate trophic state variable (Katsiapi *et al.*, 2016a).

Spearman Rank Correlation was applied to test the dependence of the five zooplankton metrics on a) total phytoplankton biovolume and b) PhyCoI. The values of the total phytoplankton biovolume and the PhyCoI index [no data for lakes Kastoria in 1999, Vegoritis 1987 and Yliki 1990, (Table 1)] from the same period with the zooplankton data were used according to Stamou *et al.* (2019a;b). Moreover, in order to evaluate the application of these metrics in assessing a) eutrophication and b) ecological water quality, the samplings were grouped based on lakes' trophic state [defined by Stamou *et al.* (2019a, b) based on mean summer phytoplankton biovol-

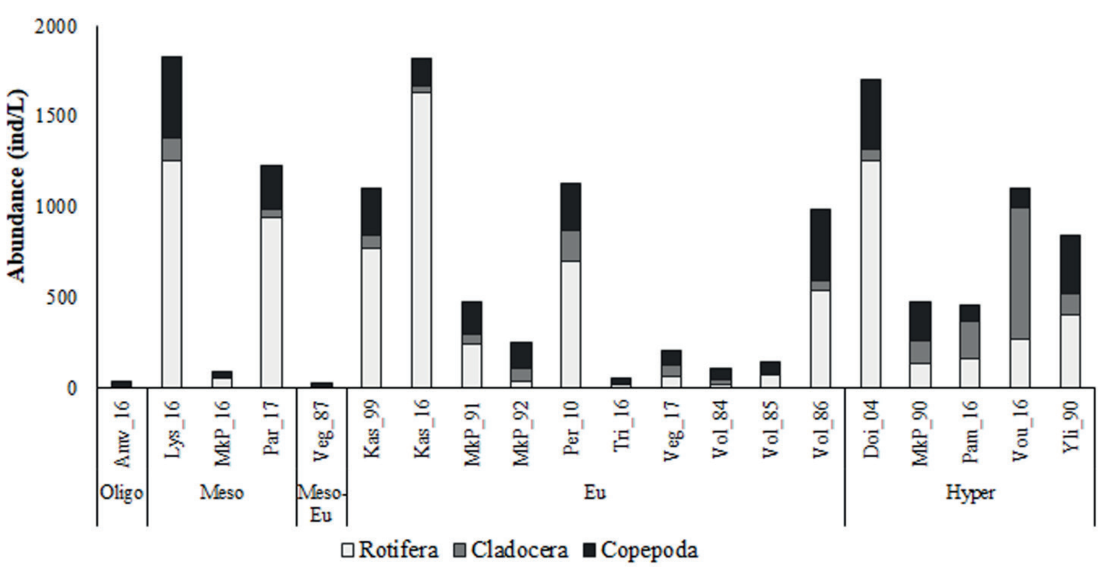

Figure 2. Mean abundance (ind/L) of the different groups of the zooplankton communities of the studied lakes across the trophic spectrum (Oligo: Oligotrophic, Meso: Mesotrophic, Meso-Eu: meso-eutrophic, Eu: Eutrophic and Hyper: Hypertrophic). Abbreviations according to figure 1 followed by year of sampling. *Abundancia media (ind/L) de los diferentes grupos de zooplancton de los lagos estudiados a lo largo del espectro trófico (Oligo: Oligotrófico, Meso: Mesotrófico, Meso-Eu: Meso-eutrófico, Eu: Eutrófico, y Hyper: Hypertrófico). Abreviaturas de los nombres de los lagos de acuerdo a la figura 1 seguido por el año de muestreo.*

ume] and lakes' ecological water quality [according to PhyCoI index according to Stamou *et al.* (2019b)] (Table 1). Probability weight for each parameter (the inverse of the sampling fraction of each category) were used to reduce bias due to the different number of samplings in each group. Welch's one-way ANOVA and the Games-Howell post-hoc test were applied to reveal whether the five zooplankton metrics differentiated among the groups of trophic state and ecological water quality. Log-transformed data [log(x+1)] were used and Welch's one-way ANOVA was applied since data conformed the normality assumption but failed the homogeneity assumption. The box-plots were drawn using non-transformed data. The above analyses were performed using IBM SPSS Statistics 25.

RESULTS

Zooplankton community structure

The mean abundance of each warm period of the zooplankton community in the 13 Greek studied lakes ranged from 27.08 ind/L (Lake Vegoritis, 1987) to 1824.94 ind/L (Lake Lysimachia) (Fig. 2). Rotifers had the highest contribution in 11 cases and their abundance ranged from 2.54 ind/L (Lake Amvrakia) to 1631.69 ind/L (Lake Kastoria, 2016); cladocerans had the highest contribution only in lakes Pamvotis and Voulkaria and their abundance ranged from 5.73 ind/L (Lake Vegoritis, 1987) to 723.64 ind/L (Lake Voulkaria); copepods had the highest contribution in 7 cases and their abundance ranged from 17.32 ind/L (Lake Vegoritis, 1987) to 444.59 ind/L (Lake Lysimachia) (Fig. 2).

The mean biomass of each warm period of the zooplankton community of the 16 lakes ranged from 29.76 μg/L (Lake Mikri Prespa, 2016) to 367.90 μg/L (Lake Voulkaria) (Fig. 3). Copepods had the highest contribution in 16 cases and their biomass ranged from 25.29 μg/L (Lake Mikri Prespa, 2016) to 200.29 μg/L (Lake Mikri Prespa, 1990); cladocerans had the highest contribution in 4 cases (Lake Kastoria in 2016, Lake Vegoritis

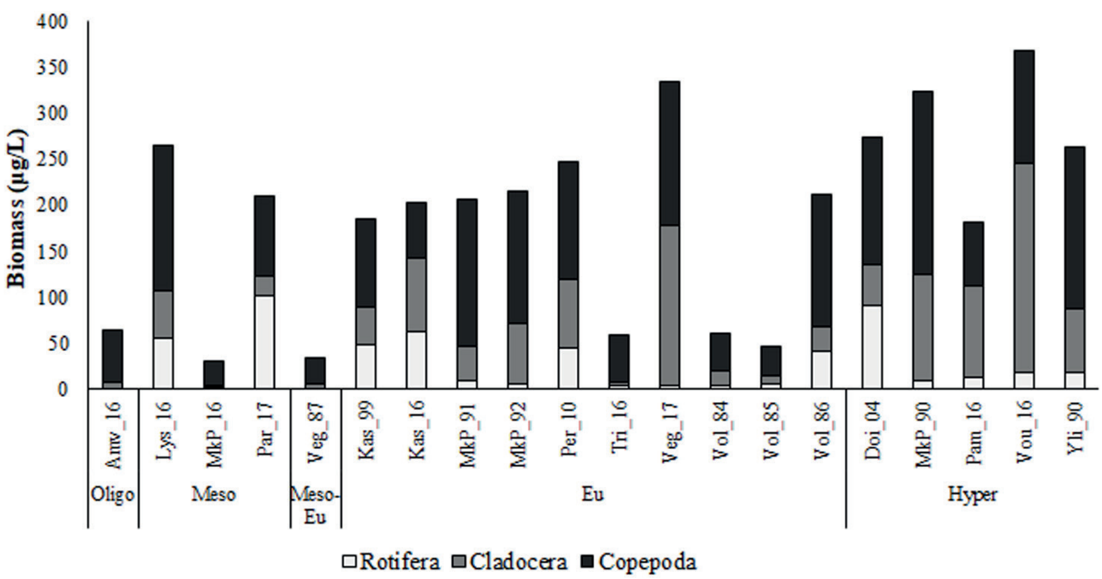

Figure 3. Mean biomass (μg/L) of the different groups of the zooplankton communities of the studied lakes across the trophic spectrum (Oligo: Oligotrophic, Meso: Mesotrophic, Meso-Eu: Meso-eutrophic, Eu: Eutrophic and Hyper: Hypertrophic). Lake abbreviations according to figure 1 followed by year of sampling. *Biomasa media (μg/L) de los diferentes grupos de zooplancton en las comunidades de los lagos estudiados a lo largo del espectro trófico (Oligo: Oligotrófico, Meso: Mesotrófico, Meso-Eu: Meso-eutrófico, Eu: Eutrófico y Hyper: Hypertrófico). Abreviaturas de los nombres de los lagos de acuerdo a la figura 1 seguido por el año de muestreo.*

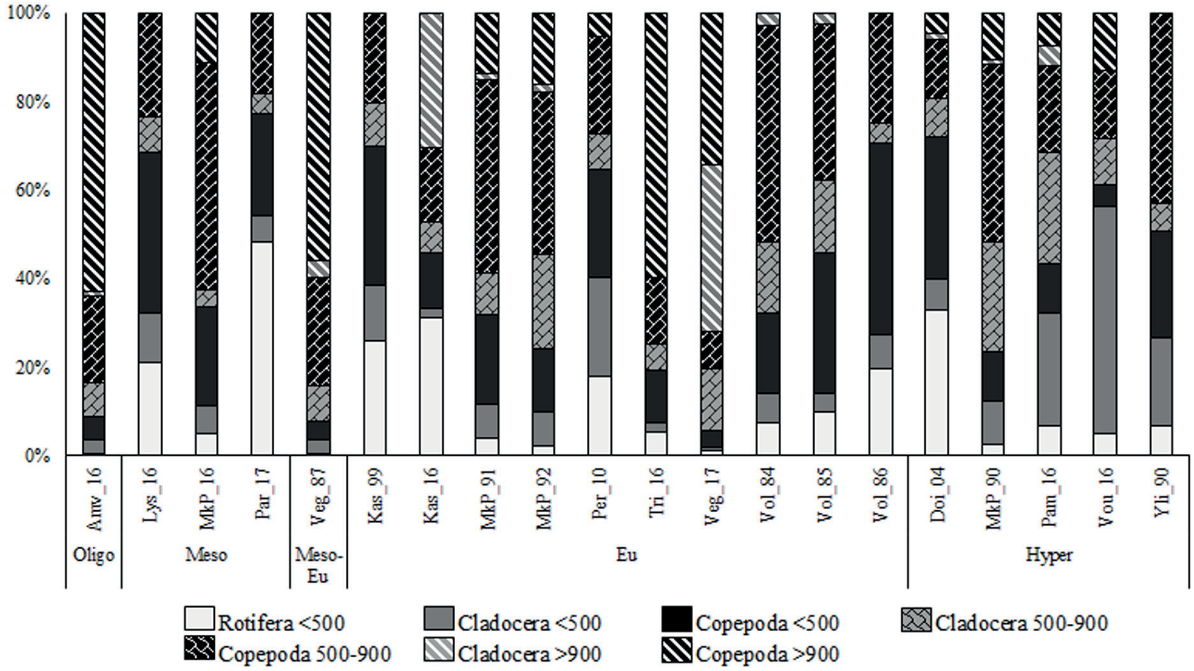

Figure 4. Contribution (%) of the size classes of the main zooplankton groups in the studied lakes across the trophic spectrum (Oligo: Oligotrophic, Meso: Mesotrophic, Meso-Eu: meso-eutrophic, Eu: Eutrophic and Hyper: Hypertrophic). Lake abbreviations according to figure 1 followed by year of sampling. *Contribución (%) de las clases de tamaño de los principales grupos de zooplancton de los lagos estudiados a lo largo del espectro trófico (Oligo: Oligotrófico, Meso: Mesotrófico, Meso-Eu: Meso-eutrófico, Eu: Eutrófico y Hyper: Hypertrófico). Abreviaturas de los nombres de los lagos de acuerdo a la figura 1 seguido por el año de muestreo.*

Table 2. Zooplankton metrics used for ecological water quality assessment, along with their description, their hypothesized response to degradation and the Reference proposing the metric. *Métricas del zooplancton usadas para la evaluación de la calidad ecológica de la masa de agua, junto con sus descripciones, su respuesta hipotética a la degradación y la referencia que propone la métrica.*

Zooplankton Metrics	**Description**	**Hypothesized response to degradation**	**Reference**
Zooplankton abundance $\mathbf{A_{Zoo}}$	Total abundance of Rotifera, Cladocera and Copepoda (in individual /L)	Increase - high values indication of eutrophic conditions	García-Chicote *et al.* (2018)
Zooplankton biomass $\mathbf{B_{Zoo}}$	Total dry biomass of Rotifera, Cladocera and Copepoda (in μg/L)	Increase - high values indication of eutrophic conditions	Jeppesen *et al.* (2011)
Zooplankton ratio $\mathbf{R_{Zoo}}$	Abundance of (Calanoida/(Cladocera + Cyclopoida) (in individual/L)	Decrease - low values indication of eutrophic conditions	Kane *et al.* (2009)
Mean zooplankton size $\mathbf{MW_{Zoo}}$	Dry biomass/abundance (in μg/individual)	Decrease - low values indicate both fish predation and eutrophication	Kane *et al.* (2009); Caroni & Irvine (2010); Jeppesen *et al.* (2011)
Cladocerans ratio $\mathbf{R_{Clad}}$	Abundance of Large-bodied cladocerans (>500 μm) /total cladocerans	Decrease - low values indicate both fish predation and eutrophication	Moss *et al.* (2003)

in 2017, Lake Pamvotis and Lake Voulkaria) and their biomass ranged from 2.97 μg/L (Lake Mikri Prespa, 2016) to 227.14 μg/L (Lake Voulkaria); finally, rotifers had the highest contribution only in Lake Paralimni and their biomass ranged from 0.13 μg/L (Lake Vegoritis, 1987) to 101.54 μg/L (Lake Paralimni) (Fig. 3).

Dominance over 50 % of large-bodied individuals, mean length > 900 μm, was recorded in three lakes, Amvrakia, Trichonis and Vegoritis (Fig. 4), mainly due to adult copepods and large cladocerans such as *Diaphanosoma* Fischer, 1850, *Daphnia* O. F. Mueller, 1785 and *Leptodora* Lilljeborg, 1861.

Zooplankton metrics

Five zooplankton metrics were applied to data of the warm period (June-September) from the 13 studied lakes and their mean values (± standard deviation) are given in Table 3.

All metrics were correlated with eutrophication either by having a significant correlation with phytoplankton biomass (Table 4) or by differentiating the trophic state categories according to Welch's one-way ANOVA, partially based on post-hoc test (Fig. 5).

From the five metrics only A_{Zoo}, R_{Zoo} and MW_{Zoo} were also correlated with ecological water quality by having a significant correlation with PhyCoI index (Table 4). All metrics differentiated the ecological water quality classes (Fig. 6). However, R_{Clad} did not show any significant differentiation based on post-hoc tests while the rest of them were partially differentiated among the different classes (Fig. 6).

DISCUSSION

In the present study, we examined the zooplankton community patterns of abundance, biomass and size structure of 13 Greek lakes and their use in ecological water quality assessment of natural Mediterranean lakes. The studied summer zooplankton communities exhibited a similar dominance pattern in terms of abundance with

Table 3. Mean values (± standard deviation) of the studied zooplankton metrics on data from zooplankton communities of 13 Greek lakes. Codes are based on lakes abbreviation according to figure 1 followed by year of sampling. *Valores medios (± desviación estandar) de las métricas de zooplancton estudiadas para las comunidades de zooplancton de 13 lagos griegos. Los códigos están basados en los nombres abreviados de los lagos de acuerdo a la figura 1 seguido por el año de muestreo.*

Codes	A_{Zoo}	B_{Zoo}	R_{Zoo}	MW_{Zoo}	R_{Clad}
Amv_16	38.64 ± 15.87	64.49 ± 22.72	2.29 ± 2.08	1.70 ± 0.30	0.43 ± 0.08
Doi_04	1704.12 ± 1291.67	274.37 ± 102.50	0.04 ± 0.62	0.17 ± 0.82	0.36 ± 0.07
Kas_99	1099.49 ± 354.62	184.87 ±121.07		0.16 ± 0.06	0.37 ± 0.12
Kas_16	1818.83 ± 2107.29	202.25 ± 137.21		0.21 ± 0.20	0.20 ± 0.34
Lys_16	1824.94 ± 1567.65	265.37 ± 200.60	0.04 ± 0.02	0.16 ± 0.03	0.15 ± 0.05
MkP_90	471.78 ± 131.53	324.43 ± 60.35	0.13 ± 0.04	0.75 ± 0.36	0.42 ± 0.02
MkP_91	478.54 ± 101.08	206.98 ± 44.42	0.37 ± 0.18	0.46 ± 0.20	0.29 ± 0.20
MkP_92	255.47 ± 50.09	214.93 ± 66.64	0.37 ± 0.14	0.84 ± 0.18	0.54 ± 0.09
MkP_16	85.74 ± 75.18	29.76 ± 19.55	1.15 ± 0.78	0.45 ± 0.20	0.06 ± 0.11
Pam_16	457.76 ± 476.62	180.78 ± 165.96	0.08 ± 0.07	0.43 ± 0.08	0.37 ± 0.23
Par_17	1231.94 ± 381.54	209.98 ± 41.76		0.19 ± 0.09	0.12 ± 0.20
Pet_10	1131.11 ± 261.46	247.63 ± 49.83		0.23 ± 0.07	0.17 ± 0.16
Tri_16	52.72 ± 15.21	58.45 ± 8.58	2.53 ± 1.25	1.16 ± 0.33	0.59 ± 0.16
Veg_87	27.08 ± 13.20	34.70 ± 19.20	2.34 ± 0.70	1.31 ± 0.33	0.54 ± 0.12
Veg_17	202.06 ± 41.66	333.89 ± 13.28	0.51 ± 0.42	1.69 ± 0.28	0.86 ± 0.04
Vol_84	109.40 ± 53.33	61.41 ± 40.44		0.53 ± 0.11	0.52 ± 0.08
Vol_85	148.15 ± 43.30	46.09 ± 22.84		0.30 ± 0.08	0.64 ± 0.07
Vol_86	986.65 ± 213.30	212.05 ± 5.56		0.22 ± 0.05	0.19 ± 0.05
Vou_16	1104.51 ± 1458.31	367.90 ± 492.45		0.30 ± 0.10	0.10 ± 0.13
Yli_90	843.17 ± 271.17	264.33 ± 102.68		0.31 ± 0.08	0.10 ± 0.07

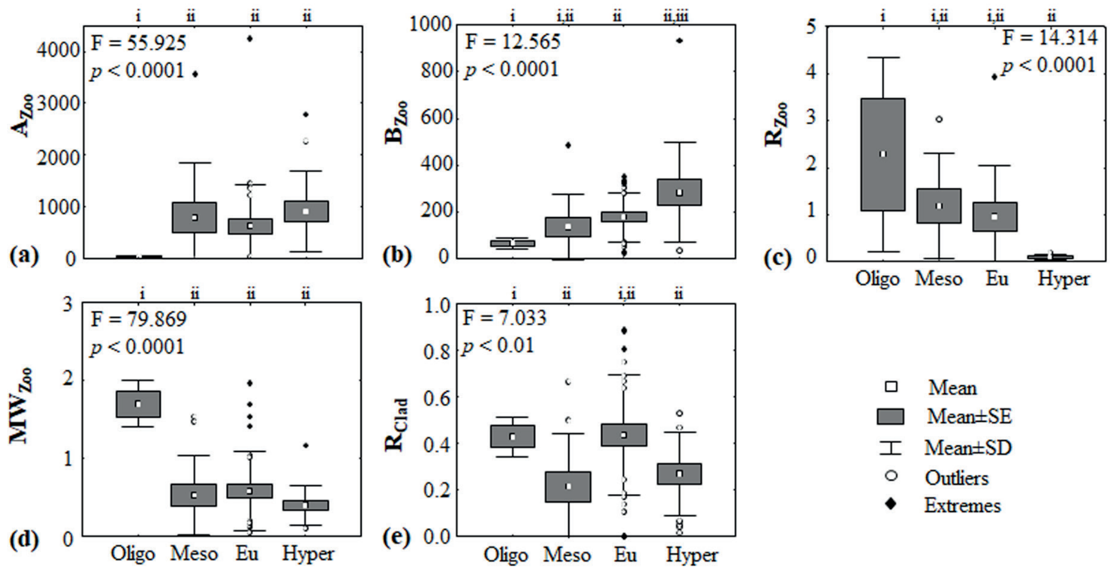

Figure 5. Box plots and Welch's one-way ANOVA analysis for the zooplankton metrics (a) A_{Zoo}, (b) B_{Zoo}, (c) R_{Zoo}, (d) MW_{Zoo} and (e) R_{Clad} grouped by trophic state (indicated by mean summer phytoplankton biomass). i, ii, iii indicate significant differences according to Games-Howell post-hoc test applied in cases with $p < 0.05$. *Diagrama de cajas y análisis ANOVA Welch de una vía para las métricas de zooplancton (a) A_{Zoo}, (b) B_{Zoo}, (c) R_{Zoo}, (d) MW_{Zoo} y (e) R_{Clad} agrupadas por estado trófico (indicado por la biomasa media de fitoplancton en verano). i, ii, iii indica diferencias significativas de acuerdo con la prueba Games-Howell post-hoc aplicada en casos con $p < 0.05$.*

mainly rotifers dominating in lakes of various trophic state. Rotifers due to their reproductive mode and their short generation time generally dominate in numbers (Allan, 1976). Under favourable environmental conditions, such as food availability, they can even reach very high numbers (Allan, 1976) as in Lake Kastoria where mean rotifers' abundance was over 1500 ind/L. Rotifers responding to bottom-up changes (Ejsmont-Karabin, 2012) can shape the total abundance of zooplankton communities. Hence, total abundance can be used as a metric of the zooplankton community reflecting rotifers contribution. The abundance metric (A_{Zoo}) has been used effectively for water quality assessment for reservoirs in the Mediterranean region (García-Chicote *et al.*, 2018) and it seems to be an appropriate metric also for natural lakes, since it was correlated with both eutrophication and ecological water quality classes increasing across degradation gradient.

Crustaceans and mainly copepods dominated in biomass, since rotifers as minute metazoans have low biomass which can be even three orders of magnitude lower compared to crustaceans (Bottrell *et al.*, 1976; Michaloudi, 2005). Biomass (B_{Zoo}) tented to increase across both trophic state and ecological water quality spectrum, although without clear differentiation for B_{Zoo} among the classes. Even though zooplankton biomass not only increases across the trophic gradient, but also decreases due to algal blooms and increased fish predation during summer (Zhao *et al.*, 2008; Ejsmont-Karabin & Karabin, 2013; Yuan & Pollard, 2018). Mediterranean lakes are characterised by both prolonged cyanobacterial blooms and increased fish predation (Vardaka *et al.*, 2005; Moustaka-Gouni *et al.*, 2014), so biomass changes can reflect either one pressure.

Besides the variation of total abundance and biomass, zooplankton ratio (R_{Zoo}) another metric based on the domination of the zooplankton groups was used. Zooplankton ratio is a functional metric based on the occurrence of the crustacean groups; calanoid copepods are more abundant in oligotrophic systems while cladocerans

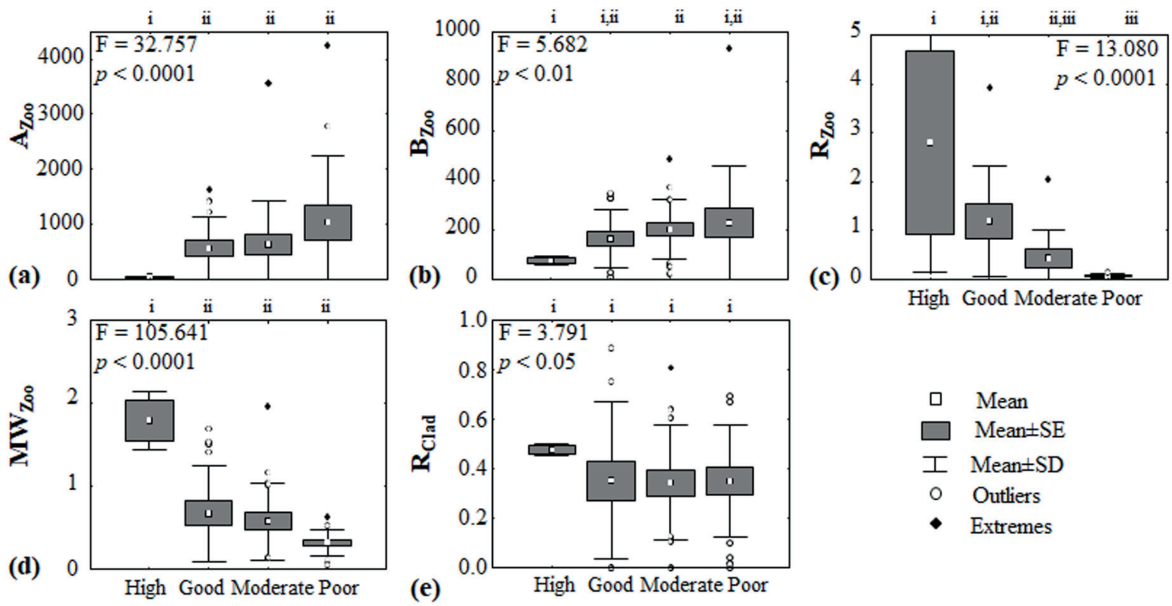

Figure 6. Box plots and Welch's one-way ANOVA analysis for the zooplankton metrics (a) A_{Zoo}, (b) B_{Zoo}, (c) R_{Zoo}, (d) MW_{Zoo} and (e) R_{Clad} grouped by ecological water quality (indicated by PhyCoI index). i, ii, iii indicate significant differences according to Games-Howell post-hoc test applied in cases with $p < 0.05$. *Diagrama de cajas y análisis ANOVA Welch de una vía para las métricas de zooplancton (a) A_{Zoo}, (b) B_{Zoo}, (c) R_{Zoo}, (d) MW_{Zoo} y (e) R_{Clad} agrupadas por calidad ecológica de la masa de agua (indicado por el índice PhyCoI). i, ii, iii indica diferencias significativas de acuerdo con la prueba Games-Howell post-hoc aplicada en casos con $p < 0.05$.*

and cyclopoid copepods dominate the eutrophic systems (e.g. Gannon & Stemberger, 1978; Karabin, 1985). R_{Zoo} was significantly correlated with both eutrophication and ecological water quality having a negative relation with lake degradation. Our dataset included past and present data for Lake Mikri Prespa, a lake showing an ecological water quality improvement (Katsiapi *et al.*, 2020) which was visible in the increase of R_{Zoo} in 2016. Thus, R_{Zoo} can be an effective tool for tracking long-term changes comparing present and historical data, as in cases where the increase of trophic state has resulted to a gradually decrease of calanoids numbers [e.g. Lake Erie (Kane *et al.*, 2009)] or even to their extinction from lake fauna as in the case of Lake Võrtsjärv (Haberman & Haldna, 2014).

Another aspect of the zooplankton community is size structure, which in the studied lakes was set by the dominant species and was influenced by phytoplankton composition and fish predation. The dominance of small-bodied species (e.g. rotifers, *Bosmina* Baird, 1845, *Chydorus* Leach, 1816) or large-bodied species (e.g. calanoid copepods, *Cyclops* O. F. Mueller, 1785, *Daphnia*) was reflected in the size classes domination pattern. For example, in lakes Pamvotis and Voulkaria the small-bodied cladocerans *Bosmina* and *Chydorus* dominated coinciding with cyanobacterial dominance (Stamou *et al.*, 2019a). These species can reach high numbers in systems with persistent cyanobacterial blooms (Vijverberg & Boersma, 1997; Hansson *et al.*, 2007). On the other hand, dominance of large-bodied species (such as daphnids and calanoids) was recorded only in the deep lakes Vegoritis, Amvrakia and Trichonis. Deep lakes favor zooplankton communities, since they can perform diel vertical migration as an avoidance measure against fish predation (Lampert, 1989; Farrell & Hodgson, 2012). This is even more important for the Mediterranean lakes, since fish predation is increased due to both the presence of omnivorous species with feeding plasticity (Bob-

Table 4. Results of Spearman Rank Correlation of the five metrics with phytoplankton biovolume and PhyCoI index. *Resultados de la correlación de rango de Spearman de las cinco métricas con el biovolumen de fitoplancton y el índice PhyCoI.*

	Phytoplankton biomass (mg/L)		PhyCoI index	
Metrics	**rho**	***p***	**rho**	***p***
A_{Zoo}	0.422	< 0.001	-0.338	< 0.05
B_{Zoo}	0.326	< 0.05	-0.215	> 0.05
R_{Zoo}	-0.462	< 0.001	0.462	< 0.001
MW_{Zoo}	-0.349	< 0.01	0.332	< 0.05
R_{Clad}	-0.15	> 0.05	0.068	> 0.05

ori *et al.*, 2013) and the extended reproductive period of fish leading to continuous numbers of fish larva (Mehner *et al.*, 1999; Meerhoff *et al.*, 2007). Thus, large-bodied zooplankton communities are indicative of both lower fish predation and increased grazing pressure to phytoplankton since high zooplankton biomass dominated by large-bodied daphnids can even lead to the clear water phase (e.g. Lampert *et al.*, 1986; Sommer *et al.*, 1986; Lathrop *et al.*, 1999).

Two metrics based on size structure were applied, the mean size of the zooplankton community (MW_{Zoo}) and the large cladocerans to total cladocerans ratio (R_{Clad}). MW_{Zoo} correlated both with eutrophication and water quality. However, R_{Clad} was not correlated significantly with ecological water quality. The ratio R_{Clad} has also been used in reservoirs at Ebro basin (Spain) without a significant correlation with ecological water quality (Montagud *et al.*, 2019). Most of the eutrophic Mediterranean freshwater systems can exhibit prolonged cyanobacterial blooms (up to eight months) (Vardaka *et al.*, 2005) which mainly affects cladocerans (e.g. Ger *et al.*, 2016). Althought, R_{Clad} did not have the expected correlation with ecological water quality in the Mediterranean systems, the monitoring of the cladoceran communities is essential. Shifts in biomass domination of different functional groups [e.g. such as daphnids, bosminids and chydorids using different feeding modes (Barnett *et al.*, 2007)] reflect changes in phytoplankton communities and provide critical information for the functioning of the food web (Jeppesen *et al.*, 2011).

According to the European policy, eutrophication is identified as a priority issue for water protection. For this metrics identifying eutrophication processes should be included in the ecological water quality assessment (European Commission, 2009). All five metrics were correlated significantly with phytoplankton biomass and or differentiated significantly among the trophic state classes. Hence, we propose all five indices to be included in a multimetric index in order to effectively assess ecological water quality combining the different potentials of each metric and their response to different pressures. Moreover, they could be incorporated to already existing plankton indices for their improvement or adaptation to different regions like a) the P-IBI based on the plankton communities of Lake Erie needing further research in order to be adapted for different regions (e.g. replacement of metrics based on specific species not inhabiting other lakes with other functional sound metrics) (Kane *et al.*, 2009) and b) $PhyCoI_{GP}$ a recently developed plankton index for the ecological water quality assessment of the Mediterranean lakes, which is mainly a phytoplankton index incorporating the Grazing Potential (GP) metric [both phytoplankton and zooplankton data are used for its calculation (Jeppesen *et al.*, 1997)], needing more zooplankton metrics in order to be a fully functional plankton index (Stamou *et al.*, 2019b).

Overall, we showed that zooplankton metrics based on abundance, biomass, body-size and groups ratios (A_{Zoo}, B_{Zoo}, R_{Zoo}, MW_{Zoo}, R_{Clad}) are promising indicators for ecological water quality assessment of Mediterranean lakes. Thus, despite of not being included as a BQE in the WFD we strongly support the use of zooplankton as a cost-efficient indicator of lake trophic state and ecological water quality, and its inclusion in monitoring programs (e.g. Caroni and Irvine 2010; Jeppesen *et al.*, 2011).

CONCLUSIONS

In conclusion, the summer zooplankton communities of the studied Mediterranean lakes followed almost the same dominance pattern with mainly rotifers dominating in abundance and crustaceans (mainly copepods) in biomass. The dominant species were influenced by phytoplankton composition (e.g. chydorids and bosminids in hypertrophic lakes dominated by cyanobacteria), while dominance of large bodied individuals over 50 % was recorded only in deep lakes. Furthermore, the five zooplankton metrics (A_{Zoo}, B_{Zoo}, R_{Zoo}, MW_{Zoo}, R_{Clad}) correlated with eutrophication and ecological water quality proving zooplankton to be an effective element for the assessment and monitoring of both eutrophication and ecological water quality using quantitative and easy to use metrics based on abundance, biomass, body-size and groups ratios.

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