

ARISTOTLE UNIVERSITY OF THESSALONIKI
FACULTY OF SCIENCE
SCHOOL OF BIOLOGY
DEPARTMENT OF ZOOLOGY

**DEVELOPMENT OF WATER QUALITY INDICES FOR MEDITERRANEAN
AQUATIC SYSTEMS BASED ON MORPHOLOGICAL, TAXONOMIC AND
FUNCTIONAL BIODIVERSITY OF ZOOPLANKTON**

PhD Dissertation

Georgia Stamou

Biologist

Thessaloniki, 2020



Operational Programme
Human Resources Development,
Education and Lifelong Learning
Co-financed by Greece and the European Union



This research is co-financed by Greece and the European Union (European Social Fund—ESF) through the Operational Programme Human Resources Development, Education and Lifelong Learning in the context of the project “Strengthening Human Resources Research Potential via Doctorate Research” (MIS-5000432), implemented by the State Scholarships Foundation (IKY).





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ΑΡΙΣΤΟΤΕΛΕΙΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΟΝΙΚΗΣ

ΣΧΟΛΗ ΘΕΤΙΚΩΝ ΕΠΙΣΤΗΜΩΝ

ΤΜΗΜΑ ΒΙΟΛΟΓΙΑΣ

ΤΟΜΕΑΣ ΖΩΟΛΟΓΙΑΣ



**ΑΝΑΠΤΥΞΗ ΔΕΙΚΤΩΝ ΠΟΙΟΤΗΤΑΣ ΝΕΡΟΥ ΜΕΣΟΓΕΙΑΚΩΝ ΥΔΑΤΙΝΩΝ
ΣΥΣΤΗΜΑΤΩΝ ΜΕ ΒΑΣΗ ΤΗ ΜΟΡΦΟΛΟΓΙΚΗ, ΤΑΞΙΝΟΜΙΚΗ ΚΑΙ
ΛΕΙΤΟΥΡΓΙΚΗ ΒΙΟΠΟΙΚΙΛΟΤΗΤΑ ΤΟΥ ΖΩΟΠΛΑΓΚΤΟΥ**

Διδακτορική διατριβή

Γεωργία Στάμου

Βιολόγος

Θεσσαλονίκη, 2020



Επιχειρησιακό Πρόγραμμα
**Ανάπτυξη Ανθρώπινου Δυναμικού,
Εκπαίδευση και Διά Βίου Μάθηση**
Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης



Το έργο συγχρηματοδοτείται από την Ελλάδα και την Ευρωπαϊκή Ένωση (Ευρωπαϊκό Κοινωνικό Ταμείο) μέσω του Επιχειρησιακού Προγράμματος «Ανάπτυξη Ανθρώπινου Δυναμικού, Εκπαίδευση και Διά Βίου Μάθηση», στο πλαίσιο της Πράξης «Ενίσχυση του ανθρώπινου ερευνητικού δυναμικού μέσω της υλοποίησης διδακτορικής έρευνας» (MIS-5000432), που υλοποιεί το Ίδρυμα Κρατικών Υποτροφιών (IKY)

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Η έγκριση της παρούσας διατριβής από το Τμήμα Βιολογίας της Σχολής Θετικών Επιστημών του Αριστοτελείου Πανεπιστημίου Θεσσαλονίκης δεν υποδηλώνει αποδοχή των γνωμών του συγγραφέως με το Ν. 5343/1932, άρθρο 202, παράγραφος 1.

Βεβαιώνω ότι είμαι συγγραφέας της παρούσας εργασίας και ότι έχω αναφέρει ή παραπέμψει σε αυτήν, ρητά και συγκεκριμένα, όλες τις πηγές από τις οποίες έκανα χρήση δεδομένων, ιδεών, προτάσεων ή λέξεων, είτε αυτές μεταφέρονται επακριβώς (στο πρωτότυπο ή μεταφρασμένες) είτε παραφρασμένες.

EXAMINATION COMMITTEE

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The PhD defence took place on the 7th of February 2020

ABSTRACT

Zooplankton community occupies an important and strategic position within the trophic web of a lake ecosystem (sandwiched between fish and phytoplankton) and is sensitive to anthropogenic impacts. Changes in zooplankton abundance, biomass and structure (dominating species and body-size) can yield information about the interactions of the pelagic communities (phytoplankton, zooplankton and fish) and food web functioning. Moreover, zooplankton due to their short generation time and their high reproductive mode, respond to eutrophication processes. Thus, it is an important element in defining the status of lake ecosystems. The aim of the present dissertation was to develop zooplankton indices based on the functional, morphological and taxonomic diversity of zooplankton groups for assessing eutrophication and ecological water quality of Mediterranean lakes during the summer period, acknowledging the particularities of the Mediterranean region. In order to do that, data of 17 Greek lakes (natural and artificial) covering the whole trophic spectrum and ecological water quality classes from poor to high were analysed (Chapter 2).

Almost all the studied summer zooplankton communities had the same dominance pattern with mainly rotifers followed by copepods developmental stage, nauplii, dominating in abundance. Biomass was dominated mainly by crustaceans (both copepods and cladocerans), while the dominant species as well as their size were influenced by phytoplankton composition and fish predation. Moreover, Copepoda were the group clustering the lakes into six groups, regardless of the lake's trophic state or morphological characteristics. According to these observations described in Chapter 3, metrics based on taxonomic, morphometric and functional traits using abundance or biomass data were selected for the development (or modification) of indices for eutrophication or ecological water quality assessment.

For eutrophication assessment (Chapter 4), two trophic state indices TSI_{ROT} and TSI_{CR} based on rotifer and crustacean communities, respectively, developed for temperate (Polish) lakes, were applied to data from the studied lakes. The evaluation of the indices was made based on the eutrophication proxies TSI_{SD} and phytoplankton biomass. Both indices increased across the eutrophication gradient, but they did not assess correctly the trophic state of the studied lakes, overestimating oligotrophic lakes and underestimating the hypertrophic ones, due to the differentiations of the plankton communities in the Mediterranean region.

Combining the whole zooplankton community, a new zooplanktonic trophic state index was proposed, TSI_{ZOO} , developed as the average of the formulae of TSI_{ROT} and TSI_{CR} which were significantly correlated with the eutrophication proxies TSI_{SD} and phytoplankton biomass. TSI_{ZOO} was proposed as the most efficient of the three zooplanktonic indices for detecting two categories of trophic state, low (oligotrophic–mesotrophic) and high (eutrophic–hypertrophic) trophic state.

For ecological water quality assessment of natural Mediterranean lakes two indices were developed, $PhyCol_{GP}$, a phytoplankton-based index incorporating Grazing potential (GP) as a metric (Chapter 5) and Zoo-IQ a zooplankton-based index (Chapter 6). Both indices can be efficiently used in ecological water quality monitoring using a five-grade classification scheme.

$PhyCol_{GP}$ is a modification of the multi-metric phytoplankton-based $PhyCol$ index developed for Greek/Mediterranean lakes including Grazing potential (GP), a functional metric measuring the potential zooplankton grazing on phytoplankton. GP is based on data of both zooplankton and phytoplankton communities incorporating weighting factors for both efficient zooplankton grazers and the suitability of phytoplankton as food. Lakes with high GP values were characterized by increased biomass dominated by large-bodied zooplankton while lakes with low GP values exhibited increased phytoplankton biomass and/or domination of small-bodied zooplankton indicating intensive fish predation. Thus, GP was incorporated to $PhyCol$ in order to enhance the phytoplankton-based ecological status assessment incorporating zooplankton as a supporting factor, as indicated by the WFD. $PhyCol_{GP}$ through the combination of six plankton metrics covering various aspects of the composition and functional structure of the plankton community successfully assessed the water quality of the studied lakes evaluated by $PhyCol$. Hence, it is proposed as a useful index for monitoring the ecological water quality of Mediterranean lakes.

The ZOOplankton Index of water Quality, Zoo-IQ, is the first index developed based only on data from zooplankton communities for water quality assessment of natural lakes in Europe. The final metrics of Zoo-IQ were abundance (A_{ZOO}), biomass (B_{ZOO}), mean zooplankton size (MW_{ZOO}) (as morphometric trait), and the ratio of large cladocerans to total cladocerans abundance (R_{Clad}) (as functional trait), all influenced by eutrophication and/or fish predation. The application of the Zoo-IQ index indicated that it successfully assessed the water quality of the studied lakes, since almost all hypertrophic lakes were characterized as poor and deep

lakes (having various trophic state) as good. Moreover, it successfully identified cases being under the implementation of restoration/biomanipulation measures. Thus, Zoo-IQ is proposed to be used in ecological water quality assessment, restoration and monitoring programs of natural Mediterranean lakes.

Overall, zooplankton proved to be an effective element for the assessment and monitoring of both eutrophication and ecological water quality and should be included as a biological quality element in the Water Framework Directive for the ecological water quality assessment.

KEYWORDS

Ecological water quality, eutrophication, Zooplankton-based index, zooplankton community structure, zooplankton metrics, TSI_{Zoo} , $PhyCol_{GP}$, Zoo-IQ, WFD, Greek lakes

ΠΕΡΙΛΗΨΗ

Η ζωοπλαγκτική κοινωνία κατέχει μια σημαντική και στρατηγική θέση στο τροφικό πλέγμα ενός λιμναίου οικοσυστήματος (μεταξύ ψαριών και φυτοπλαγκτού) και αποκρίνεται στις ανθρωπογενείς επιδράσεις. Οι αλλαγές στην αφθονία, στη βιομάζα και στη διαμόρφωση της σύνθεσης (κυρίαρχα είδη και μέγεθος) των ζωοπλαγκτικών κοινωνιών μπορούν να δώσουν πληροφορίες σχετικά με τις αλληλεπιδράσεις των πελαγικών κοινωνιών (φυτοπλαγκτού, ζωοπλαγκτού και ψαριών) και τη λειτουργία του τροφικού πλέγματος. Επιπλέον, το ζωοπλαγκτό λόγω του μικρού χρόνου γενεάς και του υψηλού ρυθμού αναπαραγωγής του, ανταποκρίνεται στις διεργασίες του ευτροφισμού. Έτσι, το ζωοπλαγκτό αποτελεί σημαντικό στοιχείο για τον καθορισμό της κατάστασης των λιμναίων οικοσυστημάτων. Σκοπός της παρούσας διατριβής ήταν η ανάπτυξη δεικτών ζωοπλαγκτού με βάση τη λειτουργική, μορφολογική και ταξινομική ποικιλότητα των ομάδων του ζωοπλαγκτού για την αξιολόγηση της τροφικής κατάστασης και της οικολογικής ποιότητας των υδάτων των μεσογειακών λιμνών κατά τη θερινή περίοδο, λαμβάνοντας υπόψη τις ιδιαιτερότητες της μεσογειακής περιοχής. Για το σκοπό αυτό αναλύθηκαν δεδομένα από 17 ελληνικές λίμνες (φυσικές και τεχνητές) που καλύπτουν το εύρος της τροφικής κατάστασης και τις κατηγορίες οικολογικής ποιότητας υδάτων από ελλιπή έως υψηλή (Κεφάλαιο 2).

Οι ζωοπλαγκτικές κοινωνίες της θερινής περιόδου που μελετήθηκαν ακολουθούσαν το ίδιο μοτίβο κυριαρχίας με τα τροχοφόρα να κυριαρχούν στην αφθονία, ακολουθούμενα από το αναπτυξιακό στάδιο των κωπηπόδων, τους ναύπλιους. Η βιομάζα κυριαρχούνταν από τα καρκινοειδή (κωπήποδα και τα κλαδοκερωτά), ενώ το κυρίαρχο είδος καθώς και το μέγεθός τους φάνηκε να καθορίζεται από τη σύνθεση του φυτοπλαγκτού και τη θήρευση των ψαριών. Επιπλέον, τα κωπήποδα ήταν η ομάδα που διαχώρισε τις λίμνες σε έξι ομάδες, ανεξάρτητα από την τροφική κατάσταση ή τα μορφολογικά χαρακτηριστικά της λίμνης. Σύμφωνα με τις παρατηρήσεις που περιγράφονται στο Κεφάλαιο 3, επιλέχθηκαν μετρικές βασισμένες σε ταξινομικά, μορφομετρικά και λειτουργικά χαρακτηριστικά χρησιμοποιώντας δεδομένα αφθονίας ή βιομάζας για την ανάπτυξη (ή τροποποίηση) δεικτών για την εκτίμηση της τροφικής κατάστασης ή της οικολογικής ποιότητας των υδάτων.

Για την εκτίμηση της τροφικής κατάστασης (Κεφάλαιο 4), δύο δείκτες ο TSI_{ROT} και ο TSI_{CR} με βάση τα τροχοφόρα και τα καρκινοειδή, αντίστοιχα, οι οποίοι αναπτύχθηκαν για εύκρατες λίμνες (της Πολωνίας), εφαρμόστηκαν στα δεδομένα των λιμνών που μελετήθηκαν

στο πλαίσιο της παρούσας έρευνας. Για την αξιολόγηση των δεικτών, οι δείκτες συσχετίστηκαν με μετρικές του ευτροφισμού, δηλαδή το δείκτη TSI_{SD} και τη φυτοπλαγκτική βιομάζα. Οι δύο δείκτες αυξάνονταν με την αύξηση της τροφικής κατάστασης, αλλά δεν εκτίμησαν σωστά την τροφική κατάσταση των εξεταζόμενων λιμνών, υπερεκτιμώντας τις ολιγότροφες λίμνες και υποτιμώντας τις υπερεύτροφες, λόγω των διαφοροποιήσεων των πλαγκτικών κοινωνιών στην περιοχή της Μεσογείου. Συνδυάζοντας μετρικές από όλη την κοινωνία του ζωοπλαγκτού, προτάθηκε ένας νέος ζωοπλαγκτικός δείκτης τροφικής κατάστασης, ο TSI_{ZOO} , που υπολογίστηκε ως ο μέσος όρος των εξισώσεων των TSI_{ROT} και TSI_{CR} που συσχετίστηκαν σημαντικά με τις μετρικές του ευτροφισμού. Από τους τρεις ζωοπλαγκτικούς δείκτες ο TSI_{ZOO} ήταν ο πιο αποτελεσματικός αναγνωρίζοντας δύο κατηγορίες τροφικής κατάστασης, της χαμηλής (ολιγότροφης-μεσότροφης) και της υψηλής (εύτροφης-υπερεύτροφης).

Για την αξιολόγηση της οικολογικής ποιότητας των φυσικών λιμνών της Μεσογείου αναπτύχθηκαν δύο δείκτες, ο $PhyCol_{GP}$, ένας δείκτης βασισμένος στο φυτοπλαγκτό που ενσωματώνει το δυναμικό βόσκησης (GP) ως μετρική (κεφάλαιο 5) και ο $Zoo-IQ$, ένας δείκτης βασισμένος στο ζωοπλαγκτό (Κεφάλαιο 6). Και οι δύο δείκτες μπορούν να χρησιμοποιηθούν αποτελεσματικά στην παρακολούθηση της οικολογικής ποιότητας των υδάτων χρησιμοποιώντας ένα σύστημα ταξινόμησης πέντε επιπέδων.

Ο $PhyCol_{GP}$ είναι μια τροποποίηση του πολυ-μετρικού δείκτη $PhyCol$, που βασίζεται στο φυτοπλαγκτό ο οποίος αναπτύχθηκε για τις ελληνικές/μεσογειακές λίμνες, συμπεριλαμβάνοντας το δυναμικού βόσκησης (GP), μια λειτουργική μετρική που εκτιμά τη βόσκηση του ζωοπλαγκτού στο φυτοπλαγκτό. Το GP βασίζεται σε δεδομένα και του ζωοπλαγκτού και του φυτοπλαγκτού τα οποία παίρνουν συντελεστές βαρύτητας τόσο για την αποτελεσματικότητα των ζωοπλαγκτικών οργανισμών ως βοσκητές όσο και για την καταλληλότητα του φυτοπλαγκτού ως τροφή. Οι λίμνες με υψηλές τιμές GP χαρακτηρίζονταν από αυξημένη βιομάζα με κυριαρχία μεγαλόσωμου ζωοπλαγκτού, ενώ οι λίμνες με χαμηλές τιμές GP παρουσίαζαν αυξημένη φυτοπλαγκτική βιομάζα ή/και κυριαρχία μικρόσωμου ζωοπλαγκτού που δείχνει έντονη θήρευση από τα ψάρια. Το GP ενσωματώθηκε στο $PhyCol$ για να ενισχύσει την εκτίμηση της οικολογικής κατάστασης με βάση το φυτοπλαγκτό ενσωματώνοντας το ζωοπλαγκτό ως παράγοντα υποστήριξης, όπως προτείνεται από την Οδηγία 2000/60. Ο $PhyCol_{GP}$, μέσω του συνδυασμού έχει πλαγκτικών μετρικών που καλύπτουν διάφορες πτυχές της σύνθεσης και της λειτουργικής δομής της πλαγκτικής

κοινωνίας, αξιολόγησε με επιτυχία την ποιότητα των υδάτων των λιμνών που μελετήθηκαν. Ως εκ τούτου, προτείνεται ως ένας χρήσιμος δείκτης για την παρακολούθηση της οικολογικής ποιότητας των υδάτων των μεσογειακών λιμνών.

Ο ζωοπλαγκτικός δείκτης ποιότητας των υδάτων, ο Zoo-IQ, είναι ο πρώτος δείκτης που αναπτύχθηκε αποκλειστικά από δεδομένα ζωοπλαγκτικών κοινωνιών για την αξιολόγηση της ποιότητας των φυσικών λιμνών στην Ευρώπη. Οι τελικές μετρικές του Zoo-IQ ήταν η αφθονία (A_{Zoo}), η βιομάζα (B_{Zoo}), το μέσο μέγεθος του ζωοπλαγκτού (MW_{Zoo}) (μορφομετρικό χαρακτηριστικό) και ο λόγος της αφθονίας των μεγαλόσωμων κλαδοκερωτών προς το σύνολο των κλαδοκερωτών (R_{Clad}) (λειτουργικό χαρακτηριστικό), οι οποίες επηρεάζονται από τον ευτροφισμό ή/και τη θήρευση από τα ψάρια. Η εφαρμογή του δείκτη Zoo-IQ έδειξε ότι αξιολόγησε με επιτυχία την ποιότητα των υδάτων των λιμνών που μελετήθηκαν, αφού σχεδόν όλες οι υπερεύτροφες λίμνες χαρακτηρίστηκαν ως ελλιπείς και οι βαθιές λίμνες (που ανήκαν σε διαφορετικές κατηγορίες τροφικής κατάστασης) ως καλές. Ακόμα, αναγνώρισε με επιτυχία περιπτώσεις που ήταν υπό την εφαρμογή μέτρων αποκατάστασης/βιοδιαχείρισης. Έτσι, ο Zoo-IQ προτείνεται να χρησιμοποιείται σε προγράμματα εκτίμησης της οικολογικής ποιότητας των υδάτων, αποκατάστασης και παρακολούθησης των φυσικών λιμνών της Μεσογείου.

Συμπερασματικά, αποδείχτηκε ότι το ζωοπλαγκτό είναι ένα αποτελεσματικό στοιχείο για την εκτίμηση και την παρακολούθηση τόσο του ευτροφισμού όσο και της οικολογικής ποιότητας των υδάτων και θα πρέπει να ενταχθεί ως βιολογικό στοιχείο ποιότητας στην Οδηγία 2000/60 για την εκτίμηση της οικολογικής ποιότητας των υδάτων.

ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ

Οικολογική ποιότητα υδάτων, ευτροφισμός, δείκτες βασισμένοι στο ζωοπλαγκτό, δομή της ζωοπλαγκτικής κοινωνίας, μετρικές του ζωοπλαγκτού, TSI_{Zoo} , $PhyCol_{GP}$, Zoo-IQ, Οδηγία 2000/60, Ελληνικές λίμνες

LIST OF PUBLICATIONS

(Based on the data derived from the current study)

Publications in scientific journals

- **Stamou G.**, Katsiapi M., Moustaka-Gouni M. and Michaloudi, E. (2019). Grazing potential - a functional plankton food web metric for ecological water quality assessment in Mediterranean lakes. *Water* 11 (6), 1274, DOI: <https://doi.org/10.3390/w11061274> (*Chapter 4*)
- **Stamou G.**, Katsiapi M., Moustaka-Gouni M. and Michaloudi E. (2019). Trophic state assessment based on zooplankton communities in Mediterranean lakes. *Hydrobiologia*, 844: 83–103 (*Chapter 5*)
- **Stamou G.**, Katsiapi M., Moustaka-Gouni M. and Michaloudi E. The zooplankton communities of Mediterranean lakes, during the summer period, as indicators of ecological water quality. (In preparation) (*Chapter 3 & 6*)
- **Stamou G.**, Katsiapi M., Mazaris A.D., Moustaka-Gouni M. and Michaloudi E. Zoo-IQ a zooplanktonic index of water quality for natural lakes in Mediterranean region (In preparation) (*Chapter 6*)

Announcements (International conferences)

- **Stamou G.**, Katsiapi M., Moustaka-Gouni M. and Michaloudi E. (2018) Plankton food-web- index in Mediterranean lakes: The case of grazing potential. HydromediT 2018 - 3rd International Congress on Applied Ichthyology & Aquatic Environment, Volos, Greece, 8-11 November, Book of Proceedings 399-403. (Oral presentation)
- **Stamou G.** and Michaloudi E. (2018) Rotifer Trophic State Index in Mediterranean freshwater systems. XV International Rotifer Symposium – Crossing Disciplinary Borders in Rotifer Research, El Paso, Texas, 3-9 June, Book of abstracts p. 98 (Poster presentation)

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ABBREVIATIONS

BQE	Biological quality element
CIS	Common Implementation Strategy
PEG model	Plankton Ecology Group model
P-IBI	Planktonic Index of Biotic Integrity
WFD	Water Framework Directive
WZI	Wetland Zooplankton Index
ZBI	Zooplankton Biotic Index
ZRTI	Zooplankton Reservoir Trophic Index
TSI	Trophic State Index
SD	Secchi depth
TP	Total phosphorus
chl-a	chlorophyll-a
TSI _{ROT}	Rotifer Trophic State index
TSI _{CR}	Crustacean Trophic State index
TSI _{zoo}	Zooplankton Trophic State index
Oligo	Oligotrophic
Meso	Mesotrophic
Eu	Eutrophic
Hyper	Hypertrophic
Zoo-IQ	Zooplankton index of water quality

1. General Introduction

- 1.1 Zooplankton Communities**
- 1.2 Zooplankton and Water Quality**
- 1.3 Aim**



1.1 Zooplankton Communities

Zooplankton has a pivotal role in freshwater ecosystems function as an intermediate link of the pelagic food web. It contributes to many ecosystem functions such as the transfer of primary production biomass to higher trophic levels, mainly fish (Carpenter et al. 1985, McQueen et al. 1986). In addition, zooplankton contributes to the recycling of nutrients, such as ammonia and phosphorus through excretion (Hessen 1992), while through respiration it contributes to CO₂ release into the water, which can be used by phytoplankton organisms during photosynthesis (Elser and Goldman 1991, Steinberg et al. 2000). Furthermore, zooplankton contributes to the increase of water clarity by grazing the available phytoplankton biomass (Pace and Orcutt 1981, Sommer et al. 1986).

Zooplankton communities of freshwater ecosystems are composed by microscopic invertebrates, mainly consisted of four groups; the Phylum Rotifera, the Subclass Copepoda, the Superorder Cladocera and the Kingdom of Protista. Other taxonomic groups (such as ostracods) and larvae of non-planktonic organisms (such as mollusc, polychaete and fish) are also commonly found in freshwater zooplankton communities as meroplankton organisms (Wetzel 2001), however they are not always mentioned in studies focused on zooplankton communities.

The main three groups, rotifers, cladocerans and copepods can be found in a variety of different aquatic habitats, and with a large range of salinities, with the majority of rotifers and cladocerans and 25% of copepods being freshwater (Fontaneto et al. 2006, Boxshall and Defaye 2008, Forró et al. 2008, Segers 2008). Rotifers and cladocerans are occasionally dominating due to their high reproductive rates and short life cycle (Allan 1976). They have several features in common both reproducing by cyclical parthenogenesis. For many reproductive generations, diploid females reproduce parthenogenetically. This phase is interrupted by the sexual phase which can occur only once, or up to several times through the year, even not at all (Allan 1976, Gilbert and Williamson 1983, Wetzel 2001), following an environmental stimulus occurs. The stimuli that induce the presence of males are still debated and appear to be largely specific; it can be either increased population density and unfavorable environmental conditions such as low temperatures, or optimal conditions such as high-quality food concentration. In any case, the presence of males leads to the sexual phase resulting in the production of resting (diapausing) eggs (Gilbert and Williamson 1983,

Wetzel 2001). Resting eggs exhibit increased resistance to extreme environmental conditions and can remain in the resting phase for long periods (up to decades), until conditions are again favorable (Hairston 1996, Havel and Shurin 2004). On the other hand, most copepods have a fundamentally different life cycle, having an obligatory sexual reproductive mode. Development from egg to adult occurs through pronounced metamorphosis through six naupliar and six copepodite stages (Gilbert and Williamson 1983, Wetzel 2001). The developmental phase of many cyclopoids can be interrupted in the last copepodite stage when they undergo a seasonal diapause while some calanoid species produce diapausing eggs (Gilbert and Williamson 1983, Hairston 1996).

Zooplankton have several life history characteristics (resting eggs, cryptobiosis for bdelloids, diapausing phase for copepodites) that allow them to persist in unstable environments. Moreover, zooplankton due to a wide variety of vectors via natural (flooding, wind, animals) or human-mediated mechanisms (canals, fish stocking) are able to passively disperse and invade new ecosystems (Havel and Shurin 2004). The establishment of new populations, even from only one individual, is possible under optimal environmental conditions due to the parthenogenetical reproduction and the short life cycle of rotifers and cladocerans (Havel and Shurin 2004). However, the colonization success and even the population recovery of copepods is low and is highly influenced by mate limitation, since mature male-female encounter may be too low to sustain the population (Havel and Shurin 2004, Kramer et al. 2008).

Seasonal succession of zooplankton has been studied in relation to physical environmental parameters and the interactions with phytoplankton and fish resulting in the development of the PEG (Plankton Ecology Group) model (Sommer et al. 1986, Sommer et al. 2012) based on data of cold-temperate lakes. According to this model, the population density of zooplankton increases in the first spring months due to the increased availability of edible phytoplankton and the hatching of resting eggs. At this point, the zooplankton community is dominated by small-bodied (mainly rotifers) zooplankton, which generally have a short life cycle and thus rapid population growth. Then, more efficient filter feeders (crustaceans) with a longer life cycle dominate and the available phytoplankton biomass begins to decline, due to the intensive grazing. In this phase, known as the clear-water phase, the phytoplankton consumption rate exceeds its renewal rate. The limitation of the available food resources contributes to the reduction of body weight and fertility of zooplankton. At the same time,

during early summer season, increased fish predation due to fish recruits with young of the year results in a big drop of the zooplankton biomass and a turnover with the domination of smaller species which are less vulnerable to fish predation. In autumn, reduced fish predation and food availability lead to an autumnal peak of zooplankton biomass including large-bodied individuals. In early winter, the reduction of light in combination with lower temperatures signifies a decrease in phytoplankton biomass followed by a decrease in the total abundance and biomass of zooplankton, while some cyclopoid species awake from diapause and dominate the low over-wintering zooplankton population. Since plankton communities are influenced by environmental factors and climatic characteristics, the PEG model is differentiated for other climatic regions, such as the polar, tropical (De Senerpont Domis et al. 2013) and the Mediterranean region (Moustaka-Gouni et al. 2014). Mediterranean lakes are differentiated from temperate lakes by morphometric (e.g. catchment size/lake size), hydrological (e.g. water level fluctuations, water residence time) and climatic characteristics (strong seasonality of rainfall and air temperature) (Alvarez Cobelas et al. 2005, Beklioglu et al. 2007, Moustaka-Gouni et al. 2014) resulting also to biological differences. The increased availability of solar radiation throughout the year leads to the continuous increase of phytoplankton even during late-autumn and winter months. Moreover, fish communities in the Mediterranean region are characterized by domination of omnivorous species with more frequent spawning, while the reproduction period is extended. As a result, there is longer and stronger fish predation leading to small-bodied zooplankton dominance and thus lower grazing pressure on phytoplankton especially during spring and summer (Beklioglu et al. 2007, Moustaka-Gouni et al. 2014).

1.2 Zooplankton and Water Quality

WFD

Over the last two decades, ecological water quality and integrity concern worldwide the scientific community due to the development of different legislation acts, i.e the Water Framework Directive (WFD) and the Marine Strategy Framework Directive in Europe, the Water Act in Australia, the US Clean Water Act and the South African National Water Act. They are all having as a main goal to maintain and enhance the ecological integrity of freshwater and marine ecosystems and focus on the assessment of the ecological water

quality of water bodies (Poikane et al. 2015). In Europe, the classification of the ecological status of freshwater bodies according to the WFD is defined based on biological quality elements (BQEs), i.e. phytoplankton, benthic macroinvertebrates, fish, aquatic macrophytes and phytobenthos (European Commission 2000). Zooplankton has not been included as a BQE in the WFD and this exclusion puzzled many ecologists, while no well-argued scientific explanation exists for its omission (e.g. Moss 2007, Caroni and Irvine 2010, Jeppesen et al. 2011).

Zooplankton communities due to their short generation time and their high reproductive rate can respond quickly to changes resulting by trophic cascades either through bottom-up or top-down control (Carpenter et al. 1985) and can increase the water clarity through grazing the available phytoplankton biomass (Pace and Orcutt 1981). Therefore, its value as an indicator of ecological conditions is undeniable (e.g. Caroni and Irvine 2010, Jeppesen et al. 2011). Trying to compensate to some extent for its omission of the WFD, zooplankton is included in two Common Implementation Strategies (CIS) of the WFD for a holistic lake assessment. Thus, zooplankton has been indicated as a supportive/interpretative parameter' of fish often/typically measured or sampled at the same time' (CIS No. 7) for monitoring top-down control (European Commission 2003) and zooplankton grazing, which may be influenced by other anthropogenic activities, as an additional lake specific - supporting environmental factor featuring the eutrophication impact' (CIS No. 23) (European Commission 2009). Still, zooplankton is the only biological element characterized as a supporting factor and not a BQE.

Following the omission of zooplankton from the WFD, the national authorities either reduce or even exclude zooplankton from monitoring programs all over Europe (Jeppesen et al. 2011). Prior to the WFD many countries (e.g. Austria, Denmark, Finland, the Netherlands and Norway) included zooplankton in their national monitoring programs of freshwater systems (EEA 1996) even for more than 50 years [e.g. in Estonia (lakes Peipsi and Võrtsjärv) (Haberman and Laugaste 2003), in Poland (lakes of Łe czna-Włodawa Lake District) (Adamczuk et al. 2015)], acknowledging the role of zooplankton in the food web as well as the easy and inexpensive sampling methodology and analysis. Zooplankton exclusion from monitoring programs is creating a gap of knowledge related to the function of the plankton food web. This is even more important for not well-studied systems such as the Mediterranean freshwater systems since food web patterns established from the knowledge derived from

the well-studied cold-temperate European lakes are differentiated in the Mediterranean climatic zones, as the PEG-model does (Moustaka-Gouni et al. 2014). Based on the above, it is necessary to monitor the plankton communities and their interactions in the Mediterranean region in order to understand the function of the freshwater ecosystems and then to be able to develop and evaluate management and restoration plans when needed (Jeppesen et al. 2011).

Zooplankton Indices

Zooplankton indicator value has been studied for many years (e.g. Gannon and Stemberger 1978, Gulati 1983, Jeppesen et al. 2011, Pomari et al. 2018). It has been used in providing information about water quality (Kane et al. 2009, Haberman and Haldna 2014), lakes' trophic state (Ejsmont-Karabin 2012, Ejsmont-Karabin and Karabin 2013), aquatic ecotoxicology (e.g. Sarma and Nandini 2006, Snell and Joaquim-Justo 2007, Kulkarni et al. 2013) and for discriminating anthropogenically disturbed lakes (Stamou et al. 2017). Important information on the status of aquatic systems is provided based on zooplankton taxonomy and functional groups.

Taxonomic identification is important since indicative species have been proposed for different trophic conditions for many regions (e.g. Gannon and Stemberger 1978, Pejler 1983, Karabin 1985). In addition, taxonomic indices based on the degree of taxonomic (phylogenetic) relatedness between species have recently been developed. These indices can differentiate anthropogenically disturbed regions, based on expected patterns of diversity in the wider region (Clarke and Warwick 2001). The application of these indices to zooplankton and in particular to the group of rotifers has differentiated Greek lakes with periods of increased salinity as a result of anthropogenic interventions (Stamou et al. 2017), while crustacean groups did not reflect a specific pressure in the Mediterranean region (Stamou 2016, Mancinelli et al. 2019)

Zooplankton groups have different functional roles in the pelagic food web. Copepods are active and selective predators with cyclopoids having a carnivorous or omnivorous diet (feeding on algae, nauplii and rotifers) and calanoids feeding mainly on phytoplankton species (Dussart and Defaye 2001). Cladocerans and rotifers are unspecialized feeders; the first being very efficient filter feeders (Dumont and Negrea 2002) while the majority of rotifers are suspension feeders or raptorial (Wallace et al. 2002). The zooplankton grazing pressure on

the phytoplankton can be strong when there is a co-occurrence with increased abundance, of large bodied cladocerans, mainly of the genus *Daphnia*, and copepods. These groups can effectively control the whole size spectrum of phytoplankton, while rotifers increase the grazing pressure even though they feed within the food size range of cladocerans (Sommer and Sommer 2006). Metrics developed based on either the different food preferences of each group (e.g. the percent of bacterivorous rotifers, ratio of calanoid to cyclopoids), and grazing pressure (i.e. Grazing potential and ratio of zooplankton to phytoplankton biomass) have been used to assess the trophic state of the ecosystem (e.g. Jeppesen et al. 1997, Ejsmont Karabin 2012, Ejsmont Karabin and Karabin 2013, Haberman and Haldna 2014).

The size of each group or the mean size of the zooplankton community in general is a morphological important characteristic of the zooplankton communities. According to the size-efficiency hypothesis, described by Brooks and Dodson (1965), when there is intense fish predation, small-bodied zooplankton (rotifers and small cladocerans) dominate. On the other hand, when fish predation pressure is low large-bodied zooplankton dominate, composed by more effective grazers (Hall et al. 1976). In the Mediterranean lakes it appears that the extended predation period by fish, (extended spawning season) does not allow zooplankton to acquire large body size to effectively graze the phytoplankton community (Moustaka-Gouni et al. 2014). Size can therefore provide information on both fish and phytoplankton communities and can be used as a metric for assessing ecological quality (Moss et al. 2003, Jeppesen et al. 2011).

The implementation of the WFD led to the increase of studies developing numerous indices for ecological water quality based on the different BQEs (Poikane et al. 2015) but not based on zooplankton. Despite that, one of the first schemes developed for the implementation of the WFD using all biological communities incorporating a holistic approach, the ECOFRAME (Moss et al. 2003), includes two zooplankton ratios (cladocerans ratio and zooplankton to phytoplankton biomass ratio). Moreover, crustaceans (cladocerans, copepods and ostracods) have been included in the QAELS index used for estimating the water quality of wetlands based on microcrustaceans and aquatic insects (Boix et al. 2005). Outside Europe, zooplankton has been used for water quality assessment in North America [Wetland Zooplankton Index (WZI) (Lougeed and Chow-Fraser 2002) and Planktonic Index of Biotic Integrity (P-IBI) (Kane et al. 2009)] and in South America [Zooplankton Biotic Index (ZBI) (De-Carli et al. 2019)]. Recently, the first index based on zooplankton data, the Zooplankton

Reservoir Trophic Index (ZRTI), has been developed for artificial Mediterranean systems and has been used in the Ebro Basin Authority's annual reports monitoring (Montagud et al. 2019). It is a case specific index since optimal or tolerant values are calculated for the species of the studied region according to their correlation with environmental data. This type of indices, despite of their great functionality, should be adapted when used in different regions with the calculation of new optimal values for the dominant species of each lake based on environmental variables (De-Carli et al. 2019).

1.3 Aim

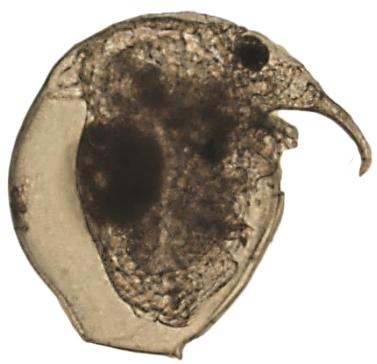
The purpose of this PhD dissertation was to develop zooplankton indices based on the functional, morphological and taxonomic diversity of zooplankton groups for assessing the ecological water quality of Mediterranean lakes during the summer period, acknowledging the particularities of the Mediterranean region.

To achieve this goal specific objectives were:

- to investigate the patterns of dominance of the summer zooplankton communities from Mediterranean lakes, through the study of natural and artificial lake zooplankton communities; contributing also to the knowledge of the biodiversity of the lakes where zooplankton communities are poorly or not at all studied (*Chapter 3, Summer Zooplankton Communities*)
- to use metrics based on the functional, morphological and taxonomic diversity of the main zooplankton groups for the assessment of eutrophication and to develop an index adapted for the Mediterranean region (*Chapter 4, Trophic State Indices*)
- to examine the sensitiveness of Grazing Potential (GP), a plankton community metric combining zooplankton and phytoplankton data, in ecological water quality changes and to include and test the GP as a metric into an existing phytoplankton index (PhyCol) so as to enhance the ecological status classification of Mediterranean lakes (*Chapter 5, Grazing Potential & PhyCol_{GP}*)
- to investigate the potentials of commonly used zooplankton metrics for the assessment of ecological water quality and to combine them into a new multi-metric zooplankton index for the assessment of the ecological water quality in five levels for

1. General Introduction

natural lakes in the Mediterranean region (*Chapter 6, Zooplanktonic Index of Water Quality*)



2. Material and Methods

- 2.1 Study area**
- 2.2 Data collection**
- 2.3 Sampling procedure**
- 2.4 Sample analysis methodology**

2.1 Study area

The present study comprised data from zooplankton communities from 17 Greek lakes (Figure 2.1); two of them, Lake Kremasta and Lake Tavropos, are reservoirs whereas the rest are natural lakes. The 17 lakes encompass a wide range of altitude, surface area, mean and maximum depth and cover the entire trophic state spectrum and the ecological water quality spectrum from poor to high (Table 2.1).

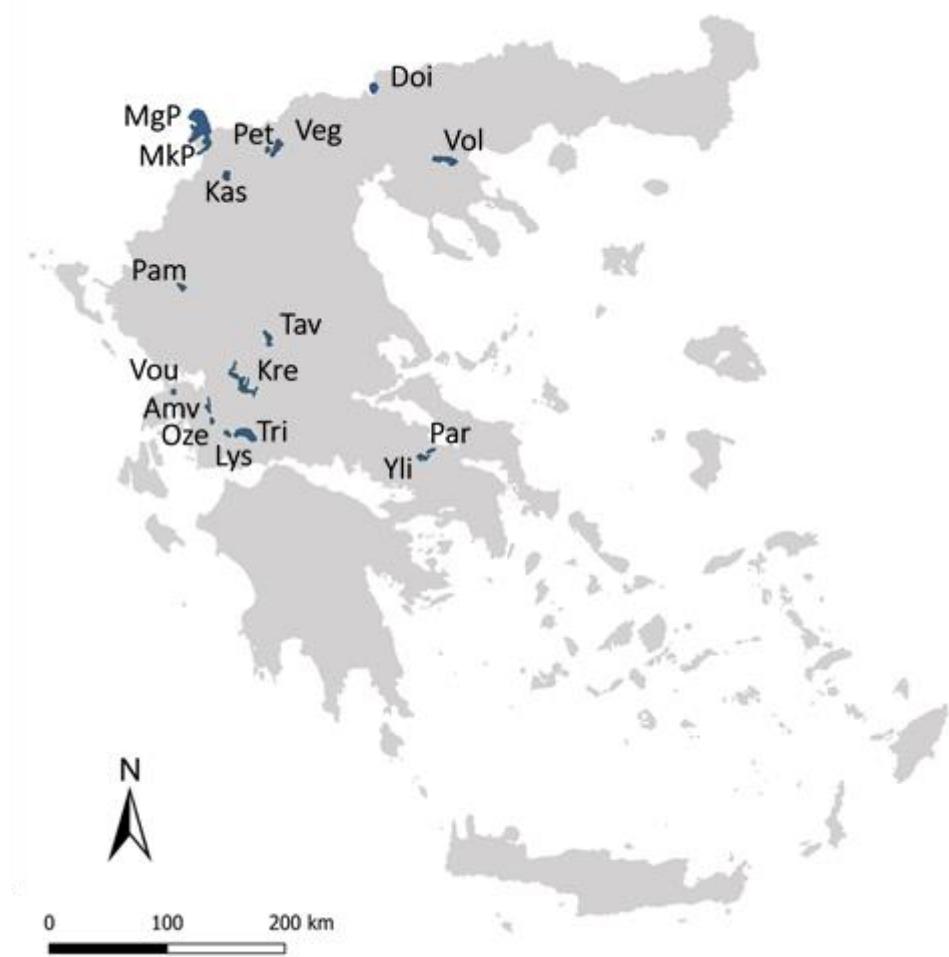


Figure 2.1 Map of Greece showing the location of the 17 lakes included in the study. Abbreviations according to Table 2.1

2. Material and Methods

Table 2.1 Topographic and morphological data, mixing type, trophic state and ecological water quality (for the respective sampling year) for the 17 studied Greek lakes. Chapter indicates the lakes data used in the analysis of the respective chapter

Lake	Abbr.	Latitude	Longitude	Surface area (Km ²)	Altitude (a.s.l.)	Mean depth (m)	Max depth (m)	Mixing type ¹	Sampling Year	Trophic state ³	Ecological Water Quality ⁴	Chapter
Amvrakia	Amv	38°45'15.60"	21°10'55.09"	14.5	25	22	53	W.M	2016	Oligo	High	3, 4, 5, 6
Doirani	Doi	41°12'55.52"	22°44'48.34"	34.8	142	3	8	P.	2004	Hyper	Poor	3, 4, 5, 6
Kastoria	Kas	40°31'09.59"	21°17'36.13"	30	629	4	9	P.	1999	Eu	n.d.	3, 4, 6
									2016	Eu	Moderate	3, 4, 5, 6
Kremasta	Kre	38°52'57.40"	21°36'04.60"	71.7	267	47	92	W.M. ²	2016	Oligo	n.d.	3, 4
Lysimachia	Lys	38°33'38.09"	21°22'22.59"	13	14.5	3.9	9	P.	2016	Meso	Good	3, 4, 5, 6
Megali Prespa	MgP	40°52'03.87"	21°01'29.31"	256.8	844	18	55	W.M.	2016	Meso-Eu	Good	3, 4, 5
Mikri Prespa	MkP	40°46'22.80"	21°05'05.96"	39.2	850	4	9	P.	1990	Hyper	Poor	3, 4, 5, 6
									1991	Eu	Moderate	3, 4, 5, 6
									1992	Eu	Moderate	3, 4, 5, 6
									2016	Meso	Good	3, 4, 5, 6
Ozeros	Oze	38°39'16.81"	21°13'32.40"	9.4	24	4.5	5.5	P.	2016	Oligo	n.d.	3, 4
Pamvotis	Pam	39°39'45.57"	20°53'27.68"	22	470	4.3	9.2	P.	2016	Hyper	Poor	3, 4, 5, 6
Paralimni	Par	38°27'53.70"	23°20'55.50"	10	51	4	8	P.	2017	Meso	Good	3, 4, 5, 6
Petron	Pet	40°43'38.79"	21°41'49.60"	11	572	3	6	P.	2010	Eu	Moderate	3, 4, 5, 6
Tavropos	Tav	39°17'39.60"	21°45'9.28"	21.6	800	>15	60	W.M. ²	1990	Oligo	n.d.	3, 4
Trichonis	Tri	38°32'47.10"	21°35'12.70"	97.2	16	30	59	W.M.	2016	Eu	Good	3, 4, 5, 6
Vegeritis	Veg	40°45'11.06"	21°47'13.76"	46	524	25	52	W.M.	1987	Meso-Eu	n.d.	3, 4, 6

2. Material and Methods

Table 2.1 Continued

Lake	Abbr.	Latitude	Longitude	Surface area (Km ²)	Altitude (a.s.l.)	Mean depth (m)	Max depth (m)	Mixing type ¹	Sampling Year	Trophic state ³	Ecological Water Quality ⁴	Chapter
Volvi	Vol	40°40'37.57"	23°28'50.61"	68.6	37	13	28	W.M.	2017	Eu	Good	3, 4, 5, 6
									1984	Eu	Moderate	3, 4, 5, 6
									1985	Eu	Poor	3, 4, 5, 6
Voulkaria	Vou	38°52'12.80"	20°50'24.20"	9.4	5	1.6	2.5	P.	2016	Hyper	Poor	3, 4, 5, 6
Yliki	Yli	38°23'56.77"	23°16'19.38"	23.0	78	21	34	W.M.	1990	Hyper	n.d.	3, 4, 6

¹W.M.: warm monomictic and P.: polymictic

²Even though they show thermal stratification they are influenced by water abstractions from hydroelectric power plants

³ Lakes' trophic state (oligo: Oligotrophic, meso: Mesotrophic, eu: Eutrophic and hyper: Hypertrophic) according to 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 PhyCol index according to Stamou et al. (2019b), n.d. no data available

2.2 Data collection

The dataset used in the present study comprised new and published data (Table 2.2). The new data were based on zooplankton samplings and the respective samples' analysis conducted for eight lakes in Western Greece in 2016, one in Central Greece (Lake Paralimni) and one in Northern Greece (Lake Vegoritis) in 2017. The published zooplankton data were collected from previous published or unpublished studies covering the summer period of previous years (1984 to 2016) (Table 2.2). These data were used, when a) zooplankton samplings and sample analysis followed the same protocols with the samples collected during the present study, b) phytoplankton data and/or secchi depth measurements of the same sampling were also available and c) three zooplankton samplings of the period June to September were available. These published data were used in order to incorporate different periods of the same lake [e.g. for lakes Mikri Prespa and Volvi different years were representing wet and dry season (Moustaka-Gouni et al. 2014)] and more recent data (e.g. lakes Mikri Prespa, Vegoritis and Kastoria)

Table 2.2 The literature source of the zooplankton and phytoplankton data used in the present study

Lake	Year	Phytoplankton data literature	Zooplankton data literature
Amvrakia	2016	Stamou et al. (2019a)	Present study
Doirani	2004	Polykarpou (2006)	Michaloudi (unpublished)
Kastoria	1999;	Moustaka-Gouni et al. (2006)	Moustaka-Gouni et al. (2006)
	2016	Stamou et al. (2019a)	Present study
Kremasta	2016	Stamou et al. (2019a)	Present study
Lysimachia	2016	Stamou et al. (2019b)	Present Study
Megali Prespa	2016	Katsiapi et al. (2016a)	Katsiapi et al. (2016a)
Mikri Prespa	1990-1992	Tryfon and Moustaka-Gouni (1997)	Michaloudi et al. (1997)
	2016	Katsiapi et al. (2016a)	Katsiapi et al. (2016a)
Ozeros	2016	Stamou et al. (2019a)	Present study
Pamvotis	2016	Stamou et al. (2019a)	Present study
Paralimni	2016	Stamou et al. (2019a)	Present study
Petron	2010	Vourka (2011)	Vourka (2011)
Tavropos	1990	Moustaka-Gouni and Nikolaidis (1992)	Moustaka-Gouni et al. (2014)
Trichonis	2016	Stamou et al. (2019a)	Present study
Vegoritis	1987	Moustaka-Gouni and Nikolaidis (1990)	Moustaka-Gouni et al. (2014)
	2017	Stamou et al. (2019a)	Present study
Volvi	1984-1986	Moustaka-Gouni (1993)	Zarfdjian et al. (1990)
Voulkaria	2016	Stamou et al. (2019a)	Present study
Yliki	1990	Moustaka-Gouni et al. (2014)	Moustaka-Gouni et al. (2014)

2.3 Sampling procedure

All samplings covered a period of 3 months during the summer period (from June to September). The summer period was chosen because this is the period used for the ecological status assessment of natural lakes in Greece/Mediterranean region [e.g. for phytoplankton (Pahissa et al. 2015, Katsiapi et al. 2016b), fish (Petriki et al. 2017)].

Zooplankton sampling was conducted in the deepest part of each lake. One integrated sample (quantitative sample) was collected from the whole water column using a 2L Niskin sampler. At least 30 L of lake water was filtered each time through a filter of mesh size 50 µm and was immediately preserved in 4% formalin (final concentration). Additional qualitative samples were collected, for species identification, with vertical and horizontal hauls using plankton nets (50 and 100 µm mesh size). These samples were analysed either while the zooplankton organisms were still alive or preserved in 4% formalin (final concentration).

During each zooplankton sampling, phytoplankton samples were also collected. Each time one integrated sample (quantitative sample) was collected from the euphotic zone (calculated as 2.5 times the Secchi depth) using a 2L Niskin sampler (Fig. 1a) and was preserved in Lugol solution and secchi depth was measured using a secchi disk.

2.4 Sample analysis methodology

Zooplankton samples were examined under a light microscope and a stereoscope. Zooplankton organisms (except mollusc larvae) were identified down to the lowest possible taxonomic level (genus or species) with the use of proper taxonomic keys, specifically for rotifers: Koste (1978), Nogrady et al. (1995), Segers (1995) and Nogrady and Segers (2002); for cladocerans: Amoros (1984), Korovchinsky (1992), Alonso (1996) and Benzie (2005); for copepods: Dussart (1967a, b), Kiefer (1968, 1971), Reddy (1994), Einsle (1996) and Dussart and Defaye (2001). Identification was performed on preserved or fresh qualitative samples and at least 400 individuals per sample were examined. All lines of taxonomic information (i.e. spellings, valid names) were confirmed for each zooplankton group [rotifers according to the Rotifer World Catalog (Jersabek and Leitner 2013) and the List of Available Names (LAN) part Rotifera (Segers et al. 2012, International Commission on Zoological Nomenclature),

2. Material and Methods

cladocerans according to the cladoceran checklist (Kotov et al. 2013) and copepods according to the World of Copepods database (Walter and Boxshall 2018)].

Abundance estimation (expressed as ind/L) was performed using the quantitative samples following the method of Bottrell et al. (1976), Downing and Rigler (1984) and Taggart (1984). In more detail, for each sample (total volume of 100 or 50 mL), five counts of 1.5 mL subsamples were made on a Sedgwick-Rafter cell using a light microscope. At least 300 individuals were counted. Otherwise when the subsample count was less than 60 individuals, the individuals of the whole sample were counted. In the case of copepods, only adult copepods were identified to species while copepodites were counted as calanoid and cyclopoid copepodites and all nauplii were grouped together.

Dry biomass ($\mu\text{g}/\text{L}$) was calculated multiplying individual dry weight values for each species for rotifers and size classes for cladocerans and copepods by the corresponding abundance value. Individual dry weight values were used according to literature or were calculated using length-dry weight relationships (e.g. Dumont et al. 1975, Ruttner-Kolisko 1977, McCauley 1984, Zarfdjian 1989, Michaloudi 2005). During the abundance estimation copepods and cladocerans size was measured (Figure 2.2) using a microscope ruler recticle in order to group them in size classes.

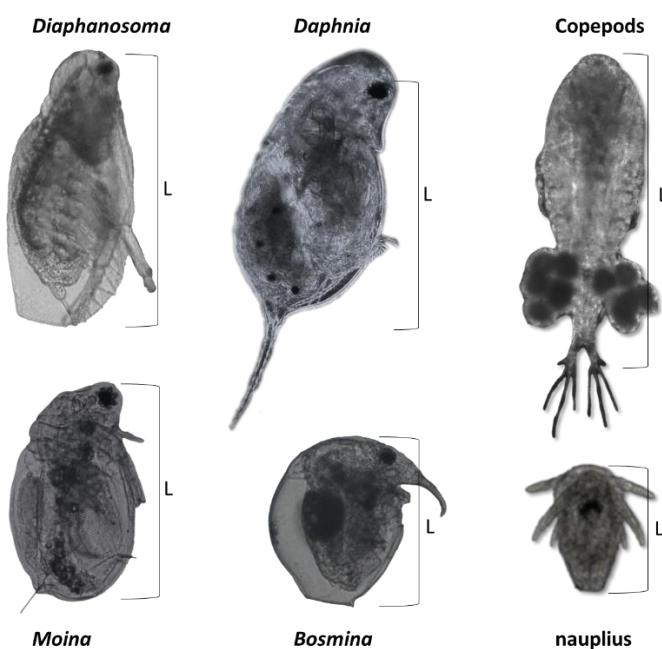
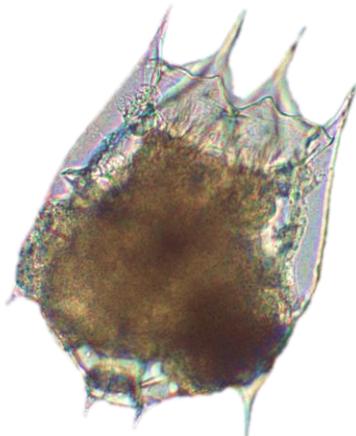


Figure 2.2 Body-length measurement of crustaceans

3. Summer Zooplankton Communities



3.1 Abstract

3.2 Introduction

3.3 Material & Methods

3.4 Results

3.5 Discussion

3.1 Abstract

The exclusion of zooplankton as a biological element from the WFD, led to the decreased monitoring of zooplankton communities all over Europe, including Greece. In the studied 17 Greek lakes covering the whole trophic spectrum, the domination patterns during the summer period were recorded. Rotifers dominated in terms of abundance, while, crustaceans, mainly small bodied species, dominated in terms of biomass. The studied lakes were clustered into six groups based on their copepod communities, regardless of the lake's trophic state or morphological characteristics. The data of the present study indicate that the dominance of the main groups and the developmental stages of other groups, such as molluscs, can give an insight in the trophic dynamics of the food web and zooplankton community structure can provide information related to the lakes' trophic characteristics and food web function.

3.1 Περίληψη

Ο αποκλεισμός του ζωοπλαγκτού ως βιολογικό στοιχείο στην Οδηγία 2000/60, οδήγησε στη μειωμένη παρακολούθηση των ζωοπλαγκτικών κοινωνιών σε όλη την Ευρώπη, συμπεριλαμβανομένης και της Ελλάδας. Στις 17 ελληνικές λίμνες που μελετήθηκαν, οι οποίες καλύπτουν όλο το εύρος τροφικής κατάστασης, καταγράφηκαν τα πρότυπα κυριαρχίας κατά τη διάρκεια της θερινής περιόδου. Τα τροχοφόρα κυριάρχησαν στην αφθονία, ενώ τα καρκινοειδή, κυρίως είδη με μικρό μέγεθος, κυριάρχησαν στη βιομάζα. Οι λίμνες που μελετήθηκαν ομαδοποιήθηκαν σε 6 ομάδες σύμφωνα με τις κοινωνίες των κωπηπόδων τους. Τα αποτελέσματα της παρούσας έρευνας δείχνουν ότι η κυριαρχία των επικρατέστερων ομάδων και τα αναπτυξιακά στάδια άλλων ομάδων όπως τα μαλάκια δίνουν μια εικόνα για τη δυναμική του τροφικού πλέγματος και η δομή της κοινότητας του ζωοπλαγκτού μπορεί να παρέχει πληροφορίες σχετικά με τα χαρακτηριστικά των λιμνών και τη λειτουργία του τροφικού πλέγματος.

3.2 Introduction

Since the implementation of the WFD, the member states had to establish a national monitoring water network for their water bodies by following the corresponding guideline documents to monitor and assess their ecological water quality. The omission of zooplankton from the WFD, led the national authorities either to reduce or even exclude zooplankton from monitoring programs all over Europe (Jeppesen et al. 2011), including Greece. This is even more crucial for Mediterranean lakes where trophic dynamics are highly affected by continuous increase of phytoplankton and extended cyanobacterial blooms and by extensive fish predation differentiating the patterns (e.g. PEG model) developed based on temperate lakes (Vardaka et al. 2005, Moustaka-Gouni et al. 2014). Given these differences, it is necessary to develop monitoring programs, including all components of the food web, that can meet the demands and spirit of the WFD, in order to develop conceptual models that can guide ecological assessment. Zooplankton is a cost-efficient indicator of lake trophic state and ecological quality, having metrics evaluating the progress of restoration plans through both top-down and bottom-up pressures (Jeppesen et al. 2011).

In Greece long data series of zooplankton communities do not exist due to the lack of a constant monitoring program for any lake. Even after the implementation of the WFD, the Greek national monitoring water network consisting of 49 lakes does not include the monitoring of zooplankton communities. During the last two decades (2000-2019) 26 publications (to the best of our knowledge-Google scholar research) exist considering zooplankton communities of only 18 Greek lakes while for 9 additional lakes only data for rotifer communities exist (Stamou et al. 2017) (Appendix 1). These datasets are derived from PhD or master thesis and short-term monitoring programs, even prior to 2000 (e.g. Kleanthidis and Sinis 2001, Michaloudi 2005) while some publications are based on already published data as part of a meta-analysis research (e.g. Mazaris et al. 2010, Moustaka-Gouni et al. 2014). This highlights the fact that the knowledge of the function of the plankton food web of Greek lakes is a combination of snapshot data for the zooplankton communities mainly of natural lakes. The current study targeted the summer zooplankton communities of 17 Greek lakes included in the Greek national monitoring water network, contributing to the existing knowledge of the zooplankton communities providing information of the patterns of zooplankton community structure (i.e. dominance of abundance and biomass, body size of

the main groups) in order to understand the function of the plankton food web and its particularities in the Mediterranean region during the period of water quality assessment for natural lakes.

3.3 Material & Methods

In order to identify the similarity of the zooplankton communities among each sampling, Hierarchical Cluster Analysis (CLUSTER) based on the Bray-Curtis similarity index was performed on the zooplankton taxa's biomass matrix using group-average linking; biomass data were log (x+1) transformed in order to reduce bias due to highly abundant groups. The similarity analysis routines, Analysis of Similarity (ANOSIM) and Similarity Percentage Analysis (SIMPER) were used to test the significance levels and sources of variance between the zooplankton assemblages of the different groups derived by the hierarchical cluster analysis. The above analyses were performed using the Plymouth Routine in Multivariate Ecological Research (PRIMER) v.6 software package (Clarke and Gorley 2006).

3.4 Results

Zooplankton communities' abundance of the 17 Greek studied lakes ranged from 9.14 ind/L (Lake Megali Prespa in July 2016) to 4245.93 ind/L (Lake Kastoria in September 2016) (Figure 3.1). In more detail, rotifers' abundance ranged from 0.63 ind/L (Lake Amvrakia in September 2016) to 3955.56 ind/L (Lake Kastoria in September 2016); cladocerans' abundance ranged from 0.67 ind/L (Lake Trichonis in September 2016) to 1943.70 ind/L (Lake Voulkatia in September 2016); copepods' abundance ranged from 2.71 ind/L (Lake Tavropos in July 1987) to 71.11 ind/L (Lake Lysimachia in August 2016); mollusc larvae's abundance ranged from 0 (45 samplings) to 782.22 ind/L (Lake Ozeros in August 2016) (Figure 3.1).

Zooplankton communities' biomass of the 17 Greek studied lakes ranged from 10.97 µg/L (Lake Mikri Prespa in July 2016) to 933.45 µg/L (Lake Voulkaria in September 2016) (Figure 3.2). In more detail, rotifers' biomass ranged from 0.02 µg/L (Lake Amvrakia in September 2016) to 169.57 µg/L (Lake Paralimni in September 2017); cladocerans' biomass ranged from 0.30 µg/L (Lake Kastoria in August 2016) to 604.93 µg/L (Lake Voulkatia in September 2016); copepods' biomass ranged from 5.97 µg/L (Lake Kremasta in September

2016) to 284.71 µg/L (Lake Lysimachia in August 2016); molluscs' biomass ranged from 0 (45 samplings) to 328.53 µg/L (Lake Ozeros in August 2016) (Figure 3.2).

Taxa comprising more than 20% of the total zooplankton abundance and biomass were considered dominant (Haberman 1976a, b). The dominating in abundance genera and developmental stages for copepods are given in Appendix 2. All cases, except lakes Amvrakia in August 2016, Kastoria in August 1999 and Pamvotis in July 2016, had at least one dominant (> 20% abundance contribution) species (or copepod's developmental stage). The higher contributions were recorded for rotifer *Filinia longiseta* in Lake Mikri Prespa (68.7% abundance contribution in July 1991) and for mollusc larvae in Lake Ozeros (68.4% abundance contribution in August 2016). The developmental stage of copepods, nauplii were dominant in 24 cases, followed by the rotifer genus *Polyarthra* dominating in 14 cases.

The dominating in biomass genera and developmental stages for copepods are given in Appendix 3. All cases, except lakes Kastoria in September 2016 and Mikri Prespa in September 1991 had at least one dominant (> 20% biomass contribution) species (or copepod's developmental stage). The higher contributions were recorded for mollusc larvae in Lake Ozeros (82.8% biomass contribution in August 2016) and *Daphnia galeata* in Lake Kastoria (68.9% biomass contribution in July 2016). The developmental stage of copepods, nauplii were dominant in 19 cases, while adult copepods of different species were dominating in 35 cases. The dominance of small-bodied species (e.g. rotifers, *Bosmina*, *Chydorus*) or large-bodied species (e.g. Calanoid copepods, *Cyclops*, *Daphnia*, *Leptodora*) was reflected at the size classes domination pattern (Figure 3.3). Dominance of large-bodied individuals over 50% was recorded in lakes Amvrakia, Kastoria, Tavropos, Trichonis and Vegeritis mainly due to adult copepods and large cladocerans such as *Leptodora*, *Daphnia* and *Diaphanosoma*.

The hierarchical cluster analysis (Figure 3.4, Table 3.1) revealed that the zooplankton community structure differed significantly between six groups of lakes (ANOSIM: $R = 0.826$, $p = 0.001$). The similarity within the groups was mainly due to the contribution of the copepod with the highest biomass (Figure 3.4, Table 3.2). Moreover, the taxa responsible for the similarity within each group were also those with the higher contribution to the dissimilarity between groups (Table 3.1). The pairwise comparison of the six groups revealed that their dissimilarity was high (SIMPER: average dissimilarity range 75-90%) (Table 3.1).

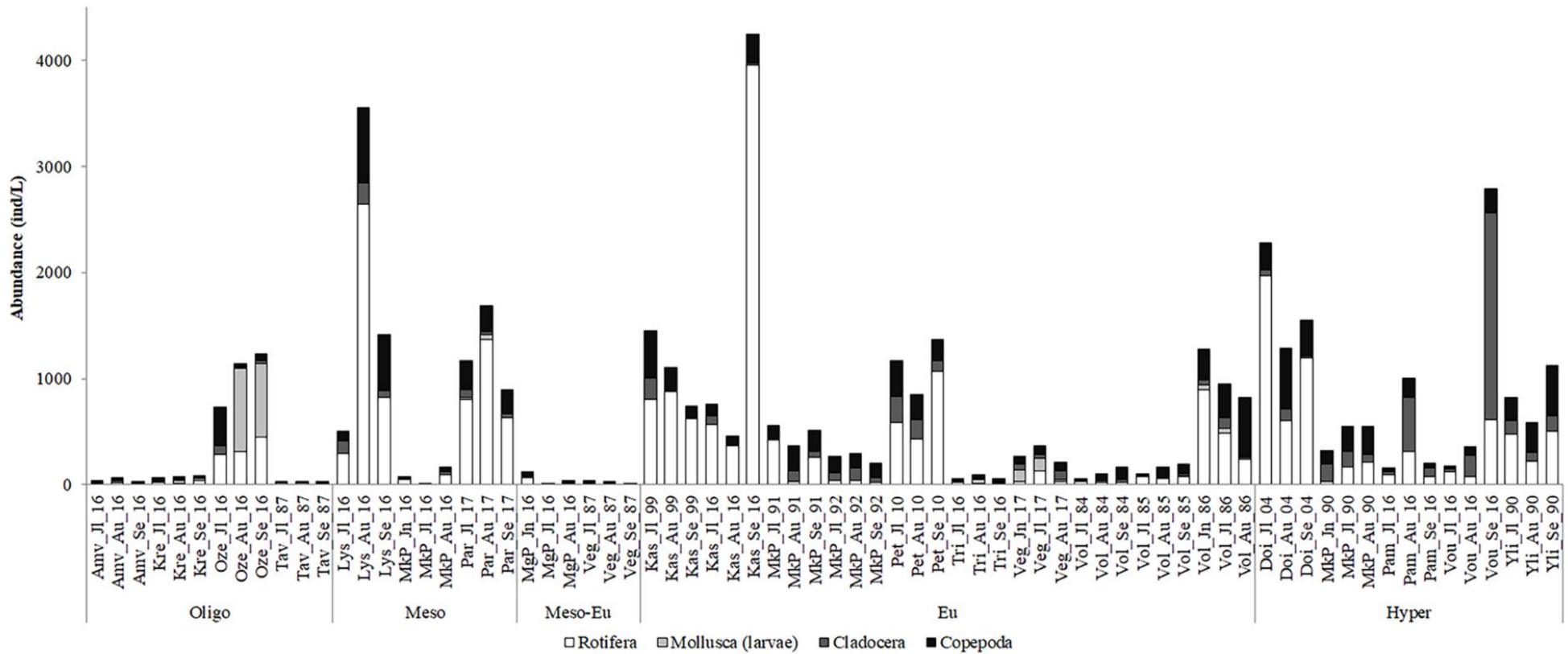


Figure 3.1 Abundance (ind/L) of the main groups of the zooplankton communities of the 17 Greek lakes across the trophic spectrum. Abbreviations according to Table 2.1 followed by month and year of sampling

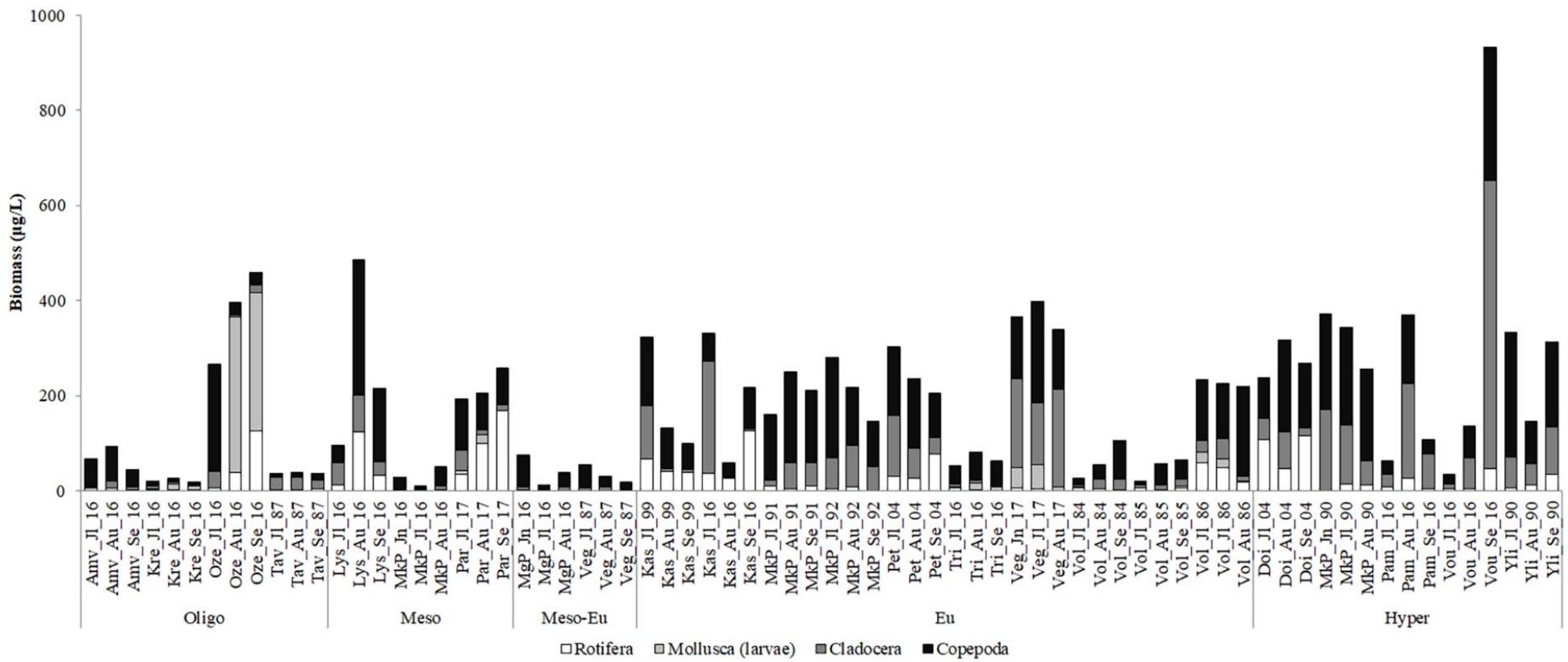


Figure 3.2 Biomass ($\mu\text{g/L}$) of the main groups of the zooplankton communities of the 17 Greek lakes across the trophic spectrum. Samplings abbreviation according to Figure 3.1

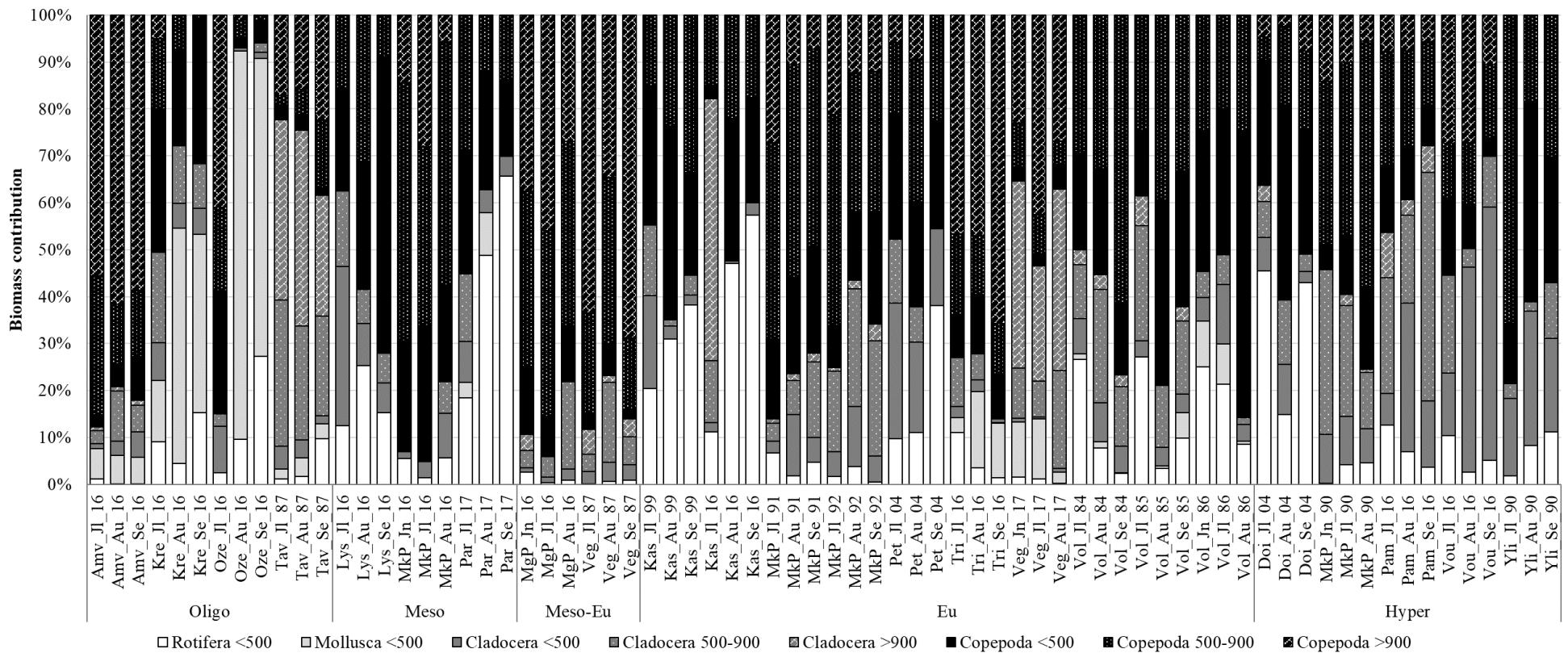


Figure 3.3 Contribution (%) of the size classes of the main zooplankton groups in 17 Greek lakes across the trophic spectrum. Samplings abbreviations according to Figure 3.1

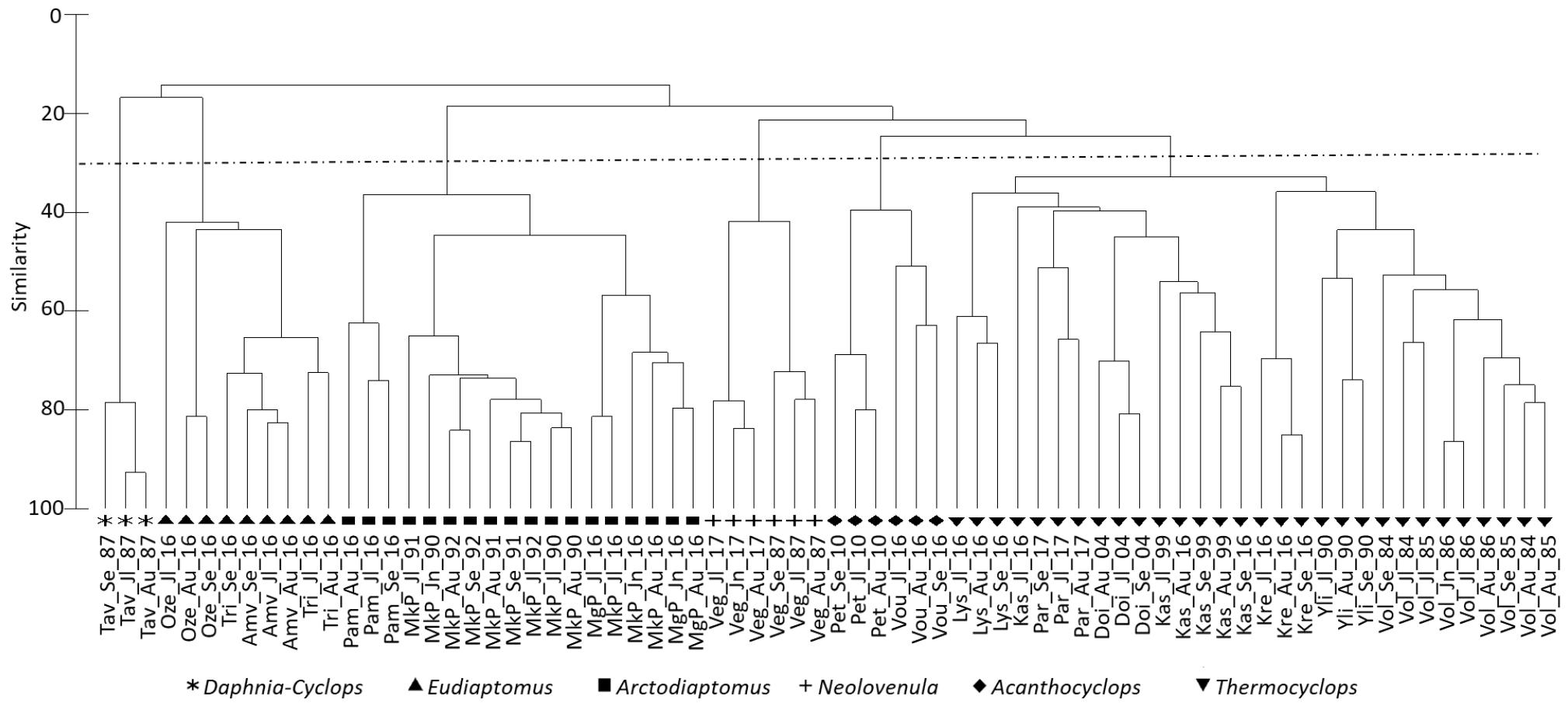


Figure 3.4 Dendrogram based on Bray-Curtis similarity index of zooplankton taxa's biomass among samplings of the 17 Greek lakes. Dashed line indicates similarity at level 30%. Symbols coded for each group based on the genera with the highest contribution to the groups' similarity. Samplings abbreviations according to Figure 3.1

3. Summer Zooplankton Communities

Table 3.1 Results of ANOSIM and SIMPER analysis applied on Bray-Curtis similarity matrix of $\log(x+1)$ transformed biomass data from zooplankton communities of 17 Greek lakes.

Groups	ANOSIM		SIMPER		
	R	Significance level %	Average dissimilarity	Most contributing to dissimilarity taxa	Contribution % (up to 20%)
<i>Eudiaptomus – Thermocyclops</i>	0.844	0.1	83.78	<i>Eudiaptomus drieschi</i> <i>Thermocyclops spp.</i>	14.24 13.24
<i>Eudiaptomus – Arctodiaptomus</i>	0.980	0.1	87.96	<i>Eudiaptomus drieschi</i> <i>Arctodiaptomus steindachneri</i>	16.44 14.96
<i>Eudiaptomus – Acanthocyclops</i>	1.000	0.3	90.59	<i>Acanthocyclops robustus</i> group <i>Eudiaptomus drieschi</i>	14.71 13.01
<i>Eudiaptomus – Daphnia-Cyclops</i>	1.000	0.5	83.16	<i>Eudiaptomus drieschi</i>	21.00
<i>Eudiaptomus – Neolovenula</i>	1.000	0.2	82.88	<i>Eudiaptomus drieschi</i> <i>Neolovenula alluaudi</i>	18.12 17.94
<i>Thermocyclops – Arctodiaptomus</i>	0.717	0.1	79.93	<i>Arctodiaptomus steindachneri</i> <i>Thermocyclops spp.</i>	13.87 13.39
<i>Thermocyclops – Acanthocyclops</i>	0.657	0.1	76.05	<i>Acanthocyclops robustus</i> group <i>Thermocyclops spp.</i>	14.26 10.13
<i>Thermocyclops – Daphnia-Cyclops</i>	0.926	0.1	86.36	<i>Thermocyclops spp.</i> <i>Daphnia spp.</i> <i>Cyclops vicinus vicinus</i>	14.58 12.95 9.98
<i>Thermocyclops – Neolovenula</i>	0.661	0.1	77.31	<i>Neolovenula alluaudi</i> <i>Thermocyclops spp.</i>	16.14 9.61
<i>Arctodiaptomus – Acanthocyclops</i>	0.912	0.1	85.03	<i>Acanthocyclops robustus</i> group <i>Arctodiaptomus steindachneri</i>	13.52 12.24
<i>Arctodiaptomus – Daphnia-Cyclops</i>	0.990	0.1	90.13	<i>Arctodiaptomus steindachneri</i> <i>Daphnia spp.</i>	16.86 14.53
<i>Arctodiaptomus – Neolovenula</i>	0.947	0.1	84.27	<i>Neolovenula alluaudi</i> <i>Arctodiaptomus steindachneri</i>	17.07 15.62
<i>Acanthocyclops – Daphnia-Cyclops</i>	1.000	1.2	89.73	<i>Acanthocyclops robustus</i> group <i>Bosmina longirostris</i>	16.69 12.70
<i>Acanthocyclops – Neolovenula</i>	1.000	0.2	87.69	<i>Acanthocyclops robustus</i> group <i>Neolovenula alluaudi</i> <i>Bosmina longirostris</i>	14.95 13.31 10.95
<i>Daphnia-Cyclops – Neolovenula</i>	1.000	1.2	85.45	<i>Neolovenula alluaudi</i>	20.22
Total	0.826	0.1			

3. Summer Zooplankton Communities

Table 3.2 Results of SIMPER analysis on Bray-Curtis similarity matrix of log(x+1) transformed biomass data from zooplankton communities of 17 Greek lakes.

Group	Average similarity	Most contributing to similarity taxa	Contribution %
<i>Acanthocyclops</i>	51.01	<i>Acanthocyclops robustus</i> group	37.20
		<i>Bosmina longirostris</i>	26.65
		<i>Diaphanosoma</i> spp.	14.07
		<i>Filinia longiseta</i>	5.28
		<i>Polyarthra</i> spp.	4.08
		<i>Asplanchna girodi</i>	2.74
<i>Arctodiaptomus</i>	51.46	<i>Arctodiaptomus steindachneri</i>	40.54
		<i>Mesocyclops leuckarti leuckarti</i>	22.42
		<i>Diaphanosoma</i> spp.	15.14
		<i>Daphnia cucullata</i>	12.49
<i>Daphnia - Cyclops</i>	83.20	<i>Daphnia</i> spp.	37.73
		<i>Cyclops vicinus vicinus</i>	27.72
		<i>Diaphanosoma</i> spp.	13.70
		<i>Mollusca</i>	8.10
		<i>Cyclopoida</i> unidentified	6.66
<i>Eudiaptomus</i>	55.43	<i>Eudiaptomus drieschi</i>	37.99
		<i>Mollusca</i>	17.68
		<i>Diaphanosoma</i> spp.	15.79
		<i>Macrocyclops albidus albidus</i>	9.03
<i>Neolovenula</i>	55.94	<i>Neolovenula alluaudi</i>	43.86
		<i>Diaphanosoma</i> spp.	18.08
		<i>Leptodora kidtnii</i>	10.64
		<i>Thermocyclops</i> spp.	10.20
		<i>Daphnia cucullata</i>	10.07
<i>Thermocyclops</i>	38.97	<i>Thermocyclops</i> sp.	37.99
		<i>Diaphanosoma</i> spp.	19.03
		<i>Polyarthra</i> spp.	11.41
		<i>Bosmina longirostris</i>	6.55
		<i>Mesocyclops leuckarti leuckarti</i>	4.56
		<i>Brachionus diversicornis</i>	4.46
		<i>Mollusca</i>	2.82
		<i>Keratella cochlearis</i>	2.28
		<i>Daphnia cucullata</i>	2.25

3.4 Discussion

In the present study, summer zooplankton communities from 17 Greek lakes included in the national monitoring program were studied, based on published and new data (samplings 2016-2017). The new data contributed to the existing knowledge (Appendix 1) with new data for lakes Amvrakia, Kastoria, Lysimachia, Ozeros, Pamvotis, Trichonis sampled in 2016 and Lake Vegeritis in 2017. Lakes Kremasta and Paralimni were monitored for the first time, while for Lake Voulkaria data for the whole zooplankton community are now available, since only data of the rotifer community of previous years have been published (Stamou et al. 2017).

The studied summer zooplankton communities followed almost the same dominance pattern with mainly rotifers followed by copepods developmental stage, nauplii dominating in abundance. Rotifers due to their reproductive mode and their short generation time generally dominate in numbers and can even reach very high numbers under favorable environmental conditions, such as food availability (Allan 1976). This was recorded in the cases of lakes Lysimachia in August 2016 and Kastoria in September 2016 when rotifers' abundance was over 2500 ind/L. However, rotifers did not dominate in biomass. 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). Therefore, biomass was dominated mainly by crustaceans (both copepods and cladocerans). The dominant species as well as their size were influenced by phytoplankton composition and fish predation. A shift in biomass dominance was recorded (also visible in size classes) from larger crustacean species to a) rotifers and b) smaller cladoceran species. For example, in the case of lakes Kastoria (2016) and Paralimni (2017) the recorded *Daphnia* and *Diaphanosoma* dominance, respectively in July was followed by the dominance of rotifers in August and September. Lake Vouklaria (2016), was a typical example for the shift to smaller cladoceran species, the dominance of *Diaphanosoma* in July was followed by the dominance of smaller cladocerans *Bosmina* and *Chydorus* in August and September, respectively, coinciding with cyanobacterial dominance (Appendix 4). This shift in the dominance pattern from larger to smaller species is expected during summer (Sommer et al. 1986) and has been attributed to predation since smaller individuals are less vulnerable to fish predation (Gliwicz and Pijanowska 1989). In Mediterranean lakes this shift is recorded even later in autumn (Fernández-Rosado and Lucena 2001) probably due to prolonged fish predation. On the other hand, dominance of large-bodied species such as daphnids and calanoids was recorded in lakes Tavropos (1987) and Vegoritis (2017) indicating increased grazing pressure to phytoplankton biomass. It is known that high zooplankton biomass dominated by large-bodied daphnids can lead to the spring clear water phase (e.g. Lampert et al. 1986, Sommer et al. 1986) and to the increased water clarity even in summer when followed by continuous dominance of large-bodied species such as daphnids and calanoids, at least in temperate lakes (Lampert et al. 1986, Lathrop et al. 1999). However, in Greek lakes even in the cases dominated by large-bodied individuals, summer zooplankton biomass was far below the threshold for producing a clear-water phase, i.e. approximately to 1.5 to 4 mg dry biomass/L (Lampert 1988), as well as

3. Summer Zooplankton Communities

zooplankton biomass throughout the year due to prolonged fish predation (Moustaka-Gouni et al. 2014). Dominance patterns and size of cladocerans during summer provide significant information on the function of the food web revealing pressures both by phytoplankton and fish and should be monitored as a proxy of the function of the food web (Jeppesen et al. 2011).

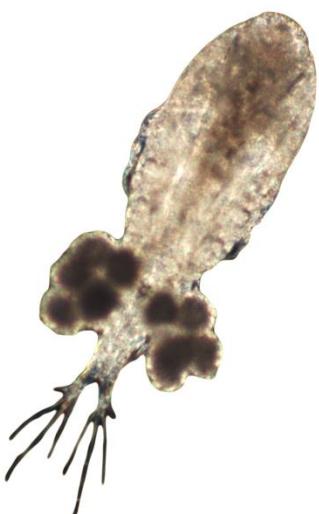
Lake Ozeros had a different pattern of dominance, since it was the only lake with a high dominance of mollusc larvae (over 60% contribution), while during the same period phytoplankton samples were dominated by detritus and exhibited low phytoplankton biomass (0.002-0.730 mg/L) indicating an oligotrophic lake. Mollusc larvae (veliger) are filter feeders of particles 1-15 µm feeding on bacterioplankton, detritus and nanoflagellates competing zooplankton for food resources especially during the summer period (Lazareva et al. 2016). Reduced phytoplankton biomass in combination with direct consumption of small zooplankton by adult molluscs might also explain low zooplankton biomass (Strayer et al. 1999, Arapov et al. 2010). Thus, the omission of mollusc in many zooplankton studies can misinterpret features of food web functioning.

Even though rotifers exhibited the highest diversity and they were also dominating in terms of abundance and species number, the studied lakes were grouped based on the biomass composition of their copepod community. Rotifers have a high dispersal ability (e.g. by different mechanisms via animal vectors, flowing surface waters) and colonization success (Cohen and Shurin 2003, Havel and Shurin 2004). Moreover, the increased number of rotifers species, even if the majority of them are present in relatively low numbers, is expected due to their high species diversity of littoral and pelagic taxa and the fine niche partitioning amongst rotifer species which is enhanced by the micro- and macroscale habitat heterogeneity (Segers 2008). On the other hand, the colonization success of copepods is low and is highly influenced by mate limitation being the only zooplankton group with obligatory sexual reproduction, which also results into lower potential for recovery after a degradation period (Kramer et al. 2008). These could explain why Copepoda is the group differentiating the studied lakes. The studied lakes were classified into six groups, with each group being dominated by the same copepod. Even in lakes inhabited by more than one copepod species (e.g. Lake Pamvotis, Lake Vegeritis, Lake Voulkaria), the copepod with the highest biomass was the most contributing taxa to the group's similarity. Hence, the studied lakes were classified into six groups based on their copepod communities, regardless of the lake's trophic

3. Summer Zooplankton Communities

state or morphological characteristics. For example, lakes Volvi, Vegoritis and Trichonis are deep natural lakes, but they were separated into three groups based on copepods community with Lake Volvi lacking calanoid copepods. Copepods are important components of the pelagic food web; cyclopoids are omnivorous feeding mainly on phytoplankton, soft-bodied rotifers and nauplii, while calanoids are herbivorous feeding mainly on various phytoplankton (Dussart and Defaye 2001) and when they co-dominate with daphnids, they enhance the grazing pressure of the entire size spectrum (Sommer and Sommer 2006).

It is evident that even though zooplankton is not a BQE for the assessment of ecological water quality, it bears attributes related to the trophic characteristics of the systems. All zooplankton components are able to show and explain differentiations among lakes, with rotifers responding immediately to environmental changes, cladocerans being important filter-feeders, mollusc larvae altering food web balance and copepods being both herbivorous and carnivorous and having low potential for recovery (e.g. Kramer et al. 2008, Arapov et al. 2010, Jeppesen et al. 2011). According to the above results, both dominance and size structure patterns changes during the warm period of the studied lakes, were influenced both by phytoplankton community dominance and fish predation. Thus, the use of relatively easy zooplankton metrics of groups dominance and the size structure should be taken into consideration in order to evaluate the food web and thus the lake functioning and they should be included in monitoring programs (Caroni and Irvine 2010, Jeppesen et al. 2011). Besides, as articulated in WFD definitions (Article 2), ecological status is an expression of the quality of the structure and functioning of aquatic ecosystems.



4. Trophic State Indices

- 4.1 Abstract
- 4.2 Introduction
- 4.3 Material & Methods
- 4.4 Results
- 4.5 Discussion

4.1 Abstract

Eutrophication classification schemes are widely based on Carlson Trophic State indices (TSI) [e.g. secchi disc depth (TSI_{SD})] or phytoplankton biomass. Recently, Carlson type trophic state indices based on zooplankton communities were developed from Polish lakes using rotifers (TSI_{ROT}) and crustaceans (TSI_{CR}). In the present study, both indices were applied to zooplankton communities from 17 Greek lakes, covering the entire trophic state spectrum, in order to test their application in the Mediterranean zone. In order to evaluate the indices (TSI_{ROT} and TSI_{CR}), the trophic state of each sampling/lake was compared with the one indicated by the eutrophication proxies TSI_{SD} (based on secchi disc) and mean summer phytoplankton biomass. Even though, both TSI_{ROT} and TSI_{CR} indices increased across the eutrophication gradient, they misclassified the trophic state underestimating mainly the eutrophic and hypertrophic lakes. A new index, TSI_{ZOO} , was developed as the average of the four formulas of TSI_{ROT} and TSI_{CR} which were significantly correlated with the eutrophication proxies. New boundaries were set in order all three zooplanktonic indices (TSI_{ROT} , TSI_{CR} and TSI_{ZOO}) to efficiently detect low (oligotrophic–mesotrophic) and high (eutrophic–hypertrophic) trophic state, i.e. <45 for TSI_{ROT} and TSI_{ZOO} and <50 for TSI_{CR} . The efficiency of the trophic indices increased when mean values of the summer season were used. All three zooplanktonic indices are effective tools for monitoring and assessment of eutrophication of Mediterranean lakes. Still, TSI_{ZOO} should be preferred in the Mediterranean region as the index with the higher correlations with eutrophication and better estimation of lakes' trophic state.

Περίληψη

Η εκτίμηση της τροφικής κατάστασης βασίζεται ευρέως στους δείκτες της Τροφικής Κατάστασης του Carlson (TSI - Trophic State Index) [π.χ. με το βάθος του δίσκου secchi (TSI_{SD})] ή στη βιομάζα φυτοπλαγκτού. Πρόσφατα, αναπτύχθηκαν δείκτες τροφικής κατάστασης τύπου Carlson με βάση τις ζωοπλαγκτικές κοινωνίες λιμνών στην Πολωνία, με βάση τα τροχοφόρα (TSI_{ROT}) και τα καρκινοειδή (TSI_{CR}). Στην παρούσα έρευνα, και οι δύο δείκτες εφαρμόστηκαν στις ζωοπλαγκτικές κοινωνίες 17 ελληνικών λιμνών, οι οποίες καλύπτουν ολόκληρο το εύρος τροφικών καταστάσεων, προκειμένου να ελεγχθεί η εφαρμογή τους στη μεσογειακή ζώνη. Για την αξιολόγηση των δεικτών (TSI_{ROT} και TSI_{CR}), η τροφική κατάσταση

κάθε δειγματοληψίας / λίμνης συγκρίθηκε με αυτή που εκτιμήθηκε από μετρικές που εκφράζουν την τροφική κατάσταση, τον δείκτη TSI_{SD} (με βάση το βάθος του δίσκου secchi) και τη μέση θερινή φυτοπλαγκτική βιομάζα. Παρόλο που οι δείκτες TSI_{ROT} και TSI_{CR} αυξάνονταν με την διαβάθμιση του ευτροφισμού, εκτίμησαν εσφαλμένα την τροφική κατάσταση υποτιμώντας κυρίως τις εύτροφες και υπερεύτροφες λίμνες. Ένας νέος δείκτης, ο TSI_{ZOO} , αναπτύχθηκε ως ο μέσος όρος των τεσσάρων εξισώσεων των TSI_{ROT} και TSI_{CR} που συσχετίστηκαν σημαντικά με τις μετρικές που εκφράζουν την τροφική κατάσταση. Δημιουργήθηκαν νέα όρια προκειμένου οι τρεις ζωοπλαγκτικοί δείκτες (TSI_{ROT} , TSI_{CR} και TSI_{ZOO}) να ανιχνεύουν αποτελεσματικά τη χαμηλή (ολιγότροφη - μεσότροφη) και υψηλή (εύτροφη - υπερεύτροφη) τροφική κατάσταση, δηλαδή <45 για τους TSI_{ROT} και TSI_{ZOO} και <50 για τον TSI_{CR} . Η αποτελεσματικότητα των δεικτών της εκτίμησης της τροφικής κατάστασης αυξήθηκε όταν χρησιμοποιήθηκαν οι μέσες τιμές της θερινής περιόδου. Και οι τρεις ζωοπλαγκτικοί δείκτες αποτελούν αποτελεσματικά εργαλεία για την παρακολούθηση και την αξιολόγηση του ευτροφισμού των μεσογειακών λιμνών. Μάλιστα, ο TSI_{ZOO} θα πρέπει να προτιμάται στην περιοχή της Μεσογείου λόγω της υψηλότερης συσχέτισης του με τον ευτροφισμό και την καλύτερη εκτίμηση της τροφικής κατάστασης των λιμνών.

4.2 Introduction

Lake eutrophication is a leading cause of impairment of many freshwater ecosystems and one of the major ecological concerns over the world in recent times. As a result of the increase of human population and the expansion of agricultural and industrial activities, freshwater systems are deteriorating worldwide with dramatic consequences, such as blooms of blue-green algae (i.e., cyanobacterial blooms), hypoxia, tainted drinking water supplies and degradation of recreational opportunities and fisheries (e.g. Carpenter et al. 1998, Smith and Schindler 2009, Callisto et al. 2014). Carlson Trophic State Index (TSI) (Carlson 1977) is one of the first and most widely used classification schemes for lakes' trophic state assessment based on Secchi depth (SD), total phosphorus (TP) and the concentration of the phytoplankton pigment chlorophyll-a (chl-a). Even though SD is used as an easy and low-cost variable of measuring water transparency, still, it is influenced both by phytoplankton abundance and non-algal particulate matter (Carlson 1977). Moreover, questionable is also the use of chl-a as a proxy of phytoplankton biomass (Kruskopf and Flynn 2006) since 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). Phytoplankton biomass estimation, being though a more laborious procedure, requiring direct counts and measurements, is considered one of the most appropriate elements for lakes' 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, Katsiapi et al. 2016b).

The same attributes can be identified in zooplankton as well. Thus, a more holistic approach, including zooplankton, should be applied for eutrophication assessment since both the structure and the function of the food web change across the eutrophication gradient (Havens 2014). For example, shallow macrophyte-dominated lakes are substantially affected by both bottom-up and top-down forces, where zooplankton grazers may limit phytoplankton biomass through top-down control (Jeppesen et al. 1997). Thus, shallow macrophyte-dominated lakes have increased possibilities for biomanipulation control of eutrophication (Beklioglu 1999), indicating that besides phytoplankton other components of the food web should also be considered critical.

Recently, two Carlson type indices have been developed using zooplankton data from Polish lakes for trophic state assessment, TSI_{ROT} based on rotifer communities (Ejsmont-Karabin 2012) and TSI_{CR} based on crustacean (cladocerans and copepods) communities (Ejsmont-Karabin and Karabin 2013). Based on the findings of other studies such as Pejler (1983) and Karabin (1985) showing that zooplankton communities tend to have a higher abundance and biomass and increased contributions of bacterivorous rotifers and cyclopoids across a eutrophication gradient Ejsmont-Karabin (2012) and Ejsmont-Karabin and Karabin (2013) developed 12 formulae (Table 4.1) based on abundance and wet biomass data of rotifer and crustacean (cladocerans and copepods) communities. Both TSI_{ROT} and TSI_{CR} are the average value of 6 formulae, and their boundaries are set as $TSI < 45$ mesotrophic, $45 < TSI < 55$ meso-eutrophic, $55 < TSI < 65$ eutrophic and $TSI > 65$ hypertrophic.

The hypothesis tested in this chapter was that the trophic state indices, TSI_{ROT} and TSI_{CR} , might differentiate and may need adaptations when applied in the Mediterranean region since mediterranean and temperate lakes are differentiated by morphometric, hydrological, climatic and biological characteristics (Alvarez Cobelas et al. 2005, Beklioglu et al. 2007, Moustaka-Gouni et al. 2014). In order to test the above hypothesis, the two indices

were applied in 17 Greek lakes along the entire trophic spectrum. The evaluation of these indices was made using the trophic state as estimated by two proxies of eutrophication, the respective TSI_{SD} and mean summer phytoplankton biomass. Furthermore, in order to propose a new TSI index based on the whole zooplankton community, namely TSI_{ZOO} , for detecting eutrophication in Mediterranean lakes, the dependence of each metric/formula of TSI_{ROT} and TSI_{CR} on eutrophication was tested. Only the significantly correlated formulae of both TSI_{ROT} and TSI_{CR} were combined into TSI_{ZOO} . Finally, the three zooplanktonic indices (TSI_{ROT} , TSI_{CR} and TSI_{ZOO}) were compared across the eutrophication gradient to find the most efficient in discriminating the trophic state in the Mediterranean region.

4.3 Material & Methods

Trophic state indices

For the assessment of lakes' trophic state, the following four trophic state indices were used on data of 17 Greek lakes (Figure 3.1, Table 2.1):

- ❖ the TSI index based on Secchi depth (TSI_{SD}) (Carlson 1977) measured during each zooplankton-phytoplankton sampling; the classification was based on the modified boundaries of Carlson and Simpson (1996).

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

- ❖ 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.
- ❖ the Rotifer Trophic State index (TSI_{ROT})
- ❖ the Crustacean Trophic State index (TSI_{CR}).

In the present study, TSI_{ROT} was estimated as the mean value of the TSI_{ROT1} , TSI_{ROT2} , TSI_{ROT3} and TSI_{ROT4} formulae (equations 2-5, Table 4.1). The formula TSI_{ROT5} for the percentage of *tecta* form in the *Keratella cochlearis* population was not used. Until recently *K. tecta* was considered a variation of *K. cochlearis* and not a separate species of the *Keratella cochlearis* species complex (Cieplinski et al. 2017). Thus, in the dataset used in the present study the abundance of *Keratella tecta* was not always recorded separately from *K. cochlearis*. The formula TSI_{ROT6} of the indicative of high trophic state rotifers was also not used since the

proposed indicator species are not appropriate for Greek lakes. For example, *Ascomorpha ecuadis* and *Gastropus stylifer* which were used by Ejsmont-Karabin (2012) as indicators of low trophic state for the respective formulae, were also found in eutrophic and even hypertrophic Greek lakes (i.e. Yliki, Mikri Prespa and Trichonis). Another example is *Pompholyx sulcata*, an indicator of high trophic state, according to Ejsmont-Karabin (2012) which was dominant in the oligotrophic lake Amvrakia (Appendix 2). Thus, this formula could not be used for Greek lakes.

TSI_{CR} in the present study was estimated as the mean value of the TSI_{CR1} , TSI_{CR2} , TSI_{CR3} and TSI_{CR4} formulae (equations 8-11, Table 4.1). The formula TSI_{CR5} for the ratio of cyclopoids to calanoids biomass was not used since it was developed only for dimictic lakes, while the studied Greek lakes were polymictic and warm monomictic (Table 2.1). The formula TSI_{CR6} of the indicator crustaceans of high trophic state was also not used. More specifically, the species *Heteropece appendiculata*, *Bosmina (Eubosmina) coregoni* and *Bythotrephes longimanus* which were proposed as indicators of low trophic state by Ejsmont-Karabin and Karabin (2013) have not been recorded in the studied Greek lakes while others i.e. *Daphnia (Daphnia) cucullata* (indicative of low trophic state) and *Bosmina (Bosmina) longirostris* (indicative of high trophic state) have been recorded in Greek lakes of the entire trophic spectrum (Appendix 2) indicating the unsuitability of the specific indicator species.

For the estimation of the trophic state for both TSI_{ROT} and TSI_{CR} indices the same boundaries were used, $TSI < 45$ mesotrophic, $45 < TSI < 55$ meso-eutrophic, $55 < TSI < 65$ eutrophic and $TSI > 65$ hypertrophic, as proposed by Ejsmont-Karabin (2012) and Ejsmont-Karabin and Karabin (2013).

Statistical analysis

Linear regression was applied in order to test the dependence of each one of the studied formulae of TSI_{ROT} and TSI_{CR} ($TSI_{ROT1} - TSI_{ROT4}$ and $TSI_{CR1} - TSI_{CR4}$) on eutrophication as estimated with the use of two proxies, TSI_{SD} and phytoplankton biomass. Considering the results of the above analyses, a new Carlson type trophic index is proposed, the Zooplankton Trophic State Index (hereafter called TSI_{ZOO}), using only the mean value of those formulae, which were significantly and strongly correlated with eutrophication. The boundaries used for lakes classification based on TSI_{ZOO} were the same used for the TSI_{ROT} and TSI_{CR} indices since it is a combination of those indices.

In order to find the more efficient of the three zooplanktonic indices, namely TSI_{ROT} , TSI_{CR} and TSI_{ZOO} , in eutrophication assessment, linear regression was applied to test the best fitted trophic index based on zooplankton data compared to the two proxies of eutrophication (TSI_{SD} and phytoplankton biomass). The same analyses were also applied to test the dependence of the three zooplanktonic indices on the percentage contribution of cyanobacteria to total phytoplankton biomass since the Mediterranean lakes exhibit prolonged cyanobacterial blooms (Vardaka et al. 2005).

Finally, 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 as indicated based only on phytoplankton biomass since it is a more reliable index compared to TSI_{SD} for Greek lakes (Katsiapi et al. 2016b). Thus, the trophic category of each lake based on mean summer phytoplankton biomass (Table 2.1) was used. The cases characterized as "meso-eutrophic" were grouped to the mesotrophic category. ANOVA and Bonferroni procedures were applied to reveal if the TSI_{ROT} , TSI_{CR} and TSI_{ZOO} indices differed among the four groups of trophic state (oligotrophic, mesotrophic, eutrophic and hypertrophic). Moreover, weight cases were used to reduce bias for each parameter due to there being different number of lakes or samplings in each group. Furthermore, a different lake classification to low (oligotrophic and mesotrophic) and high (eutrophic and hypertrophic) trophic categories was made and t-test and weight cases were also used to reveal if the TSI_{ROT} , TSI_{CR} and TSI_{ZOO} indices differed between the two groups.

All statistical analyses were performed using IBM SPSS Statistics 25.

4.4 Results

The mean summer values (\pm standard deviation) of the trophic state indices are given in Table 4.2, while the values for each sampling are given in Appendix 4. 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 Vegeritis, 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 Vegeritis, 1987).

Table 4.1 The equations of TSI_{ROT} and TSI_{CR} developed for Polish lakes by Ejsmont-Karabin (2012) and Ejsmont-Karabin and Karabin (2013), respectively. Formulae in bold are those used in the present study

Equations	Abbreviations	Comments
(2) $TSI_{ROT1} = 5.38 \ln(N) + 19.28$	N: total rotifers abundance (ind/L)	
(3) $TSI_{ROT2} = 5.63 \ln(B) + 64.47$	B: total wet rotifers biomass (mg/L)	<i>Asplanchna</i> spp. was excluded due to its larger body size compared to other rotifers excluding <i>Asplanchna</i> spp.
(4) $TSI_{ROT3} = 0.23 BAC + 44.30$	BAC: the percentage of the bacterivorous rotifers in terms of rotifer abundance	<i>Anuraeopsis fissa</i> , <i>Filinia</i> spp., <i>Brachionus angularis</i> , <i>Keratella cochlearis</i> and <i>Pompholyx sulcata</i>
(5) $TSI_{ROT4} = 3.85 (B:N)^{-0.318}$	B:N: the ratio of wet biomass to rotifera abundance (mg/ind)	
(6) $TSI_{ROT5} = 0.187 TECTA + 50.38$	TECTA: the percentage of <i>Keratella cochlearis</i> f. <i>tecta</i> individuals to the sum of <i>K. cochlearis</i> population	
(7) $TSI_{ROT6} = 0.203 IHT + 40.0$	IHT the percentage of species indicators of high trophic state to sum of species indicators	<u>Indicators of high trophic state:</u> <i>A. fissa</i> , <i>B. angularis</i> , <i>Brachionus calyciflorus</i> , <i>Filinia longiseta</i> , <i>K. cochlearis</i> f. <i>tecta</i> , <i>Keratella quadrata</i> , <i>P. sulcata</i> and <i>Trichocerca pusilla</i> . <u>Indicators of low trophic state:</u> <i>Ascomorpha ecaudis</i> , <i>Ascomorpha ovalis</i> and <i>Gastropus stylifer</i> .
(8) $TSI_{CR1} = 25.5 N^{0.142}$	N: total crustacean abundance (ind/L)	
(9) $TSI_{CR2} = 57.6 B^{0.081}$	B: total wet cyclopoids biomass (mg/L)	
(10) $TSI_{CR3} = 40.9 CB^{0.097}$	CB: Percentage of cyclopoid biomass in the total crustacean biomass	
(10) $TSI_{CR4} = 58.3 (CY/CL)^{0.071} \times$	CY/CL: Ratio of cyclopoids to cladocerans biomass	
(12) $TSI_{CR5} = 5.08 \ln(CY/CA) + 46.6$	CY/CA: Ratio of cyclopoids to calanoids biomass	
(13) $TSI_{CR6} = 43.8 e^{0.004(IHT)}$	IHT the percentage of species indicators of high trophic state to sum of species indicators	<u>Indicators of high trophic state:</u> <i>Mesocyclops leuckarti leuckarti</i> , <i>Thermocyclops oithonoides</i> , <i>Diaphanosoma brachyurum</i> , <i>Chydorus sphaericus</i> , <i>Bosmina coregoni</i> (Syn: <i>Bosmina coregoni thersites</i>), <i>Bosmina longirostris</i> . <u>Indicators of low trophic state:</u> <i>Heterocope appendiculata</i> , <i>Bosmina coregoni</i> (Syn.: <i>Bosmina berolinensis</i>), <i>Bythotrephes longimanus</i> , <i>Daphnia galeata</i> , <i>Daphnia cristata</i> and <i>Daphnia cucullata</i> .

4. Trophic State Indices

Table 4.2 Mean values (\pm standard deviation) of TSI_{SD} , phytoplankton biomass (PB) (mg/L), TSI_{ROT} and its formulae TSI_{ROT1} - TSI_{ROT4} , TSI_{CR} and its formulae TSI_{CR1} - TSI_{CR4} and TSI_{ZOO} . Codes are based on lakes abbreviation according to Table 2.1 followed by year of sampling, m.v.: missing value

Codes	TSI_{SD}	PB	TSI_{ROT1}	TSI_{ROT2}	TSI_{ROT3}	TSI_{ROT4}	TSI_{ROT}	TSI_{CR1}	TSI_{CR2}	TSI_{CR3}	TSI_{CR4}	TSI_{CR}	TSI_{ZOO}
Amv_16	33.24 \pm 4.01	0.16 \pm 0.05	22.82 \pm 5.38	26.60 \pm 10.04	52.41 \pm 2.31	42.28 \pm 16.23	36.03 \pm 3.40	42.19 \pm 2.22	47.05 \pm 1.46	52.60 \pm 1.98	59.60 \pm 4.20	50.36 \pm 1.51	34.66 \pm 4.08
Doi_04	70.70 \pm 5.77	21.04 \pm 2.23	57.07 \pm 3.20	63.47 \pm 2.83	47.10 \pm 2.78	39.09 \pm 3.47	51.43 \pm 2.63	60.21 \pm 3.66	53.83 \pm 3.13	55.81 \pm 2.57	58.84 \pm 4.35	57.17 \pm 2.79	58.65 \pm 0.42
Kas_99	78.31 \pm 2.55	13.39 \pm 3.48	54.97 \pm 0.94	60.19 \pm 1.69	52.12 \pm 4.33	40.52 \pm 3.64	51.95 \pm 1.73	56.42 \pm 6.95	54.21 \pm 1.73	58.75 \pm 1.97	63.25 \pm 5.95	58.16 \pm 0.96	56.45 \pm 2.73
Kas_16	m.v.	8.04 \pm 6.52	56.12 \pm 6.79	60.58 \pm 4.54	52.74 \pm 6.99	42.60 \pm 6.39	53.01 \pm 6.01	52.83 \pm 4.67	53.11 \pm 2.83	58.35 \pm 3.39	66.18 \pm 12.61	57.62 \pm 3.26	55.66 \pm 4.42
Kre_16	51.89 \pm 2.10	0.44 \pm 0.24	35.71 \pm 3.29	41.98 \pm 2.58	44.84 \pm 0.94	35.24 \pm 2.38	39.44 \pm 2.00	41.83 \pm 1.01	41.58 \pm 3.75	53.16 \pm 4.24	54.82 \pm 2.99	47.85 \pm 2.95	40.27 \pm 1.28
Lys_16	62.09 \pm 0.98	3.84 \pm 2.66	55.65 \pm 5.90	58.76 \pm 6.61	56.70 \pm 3.57	45.61 \pm 1.29	50.03 \pm 9.14	61.53 \pm 6.56	53.11 \pm 6.32	54.69 \pm 3.90	57.23 \pm 5.03	56.64 \pm 5.36	57.26 \pm 6.30
MgP_16	46.72 \pm 4.87	1.82 \pm 1.96	31.75 \pm 9.82	32.34 \pm 10.13	53.60 \pm 7.57	49.46 \pm 2.50	41.79 \pm 6.33	40.26 \pm 5.66	45.65 \pm 3.21	53.76 \pm 0.13	60.58 \pm 2.83	50.06 \pm 1.92	37.50 \pm 7.15
MkP_90	64.91 \pm 2.18	19.87 \pm 11.04	44.22 \pm 5.78	47.42 \pm 9.60	59.07 \pm 3.31	44.58 \pm 9.87	48.82 \pm 2.40	58.15 \pm 1.09	56.55 \pm 1.69	56.07 \pm 3.10	57.34 \pm 3.95	57.03 \pm 2.32	51.59 \pm 4.47
MkP_91	57.22 \pm 1.48	4.08 \pm 2.25	46.50 \pm 7.08	50.04 \pm 2.80	55.40 \pm 9.16	44.23 \pm 11.08	49.04 \pm 6.50	55.10 \pm 3.38	53.39 \pm 2.89	54.69 \pm 2.44	59.17 \pm 0.82	55.59 \pm 1.91	51.26 \pm 1.65
MkP_92	62.10 \pm 1.26	5.32 \pm 2.07	38.08 \pm 2.20	44.92 \pm 6.98	49.77 \pm 4.56	35.85 \pm 10.25	42.16 \pm 1.25	54.81 \pm 1.38	51.70 \pm 1.40	52.42 \pm 0.97	54.67 \pm 1.68	53.40 \pm 0.84	47.38 \pm 2.76
MkP_16	53.14 \pm 3.11	1.20 \pm 0.54	37.24 \pm 8.51	37.85 \pm 8.54	56.41 \pm 2.95	50.06 \pm 1.44	45.39 \pm 4.99	41.11 \pm 5.09	44.69 \pm 6.40	53.10 \pm 4.61	59.77 \pm 2.68	49.67 \pm 4.25	40.22 \pm 7.03
Oze_16	47.12 \pm 8.29	0.27 \pm 0.40	50.68 \pm 1.26	57.97 \pm 8.33	44.30 \pm 0	37.38 \pm 16.04	47.58 \pm 1.76	51.04 \pm 8.55	24.85 \pm 21.52	25.29 \pm 22.15	29.59 \pm 25.59	32.67 \pm 18.77	46.14 \pm 6.83
Pam_16	82.65 \pm 9.15	16.44 \pm 3.14	45.69 \pm 4.00	51.13 \pm 4.00	48.93 \pm 3.79	38.99 \pm 3.68	46.19 \pm 0.90	53.64 \pm 9.76	51.85 \pm 5.10	54.94 \pm 2.17	54.26 \pm 2.16	53.67 \pm 4.34	50.58 \pm 5.84
Par_17	60.20 \pm 2.93	2.20 \pm 1.69	55.81 \pm 2.11	63.50 \pm 4.49	46.52 \pm 2.85	36.23 \pm 10.01	50.52 \pm 2.33	57.13 \pm 1.18	54.25 \pm 1.89	59.22 \pm 1.57	62.76 \pm 2.54	58.34 \pm 0.93	57.67 \pm 0.53
Pet_10	65.29 \pm 3.59	9.83 \pm 1.17	54.09 \pm 2.49	59.19 \pm 3.37	50.89 \pm 3.49	40.44 \pm 2.17	51.15 \pm 1.96	60.16 \pm 2.92	56.74 \pm 1.71	58.90 \pm 2.37	59.38 \pm 2.92	58.80 \pm 1.24	57.55 \pm 0.62
Tav_87	47.43 \pm 1.55	0.19 \pm 0.10	25.64 \pm 4.57	38.26 \pm 6.60	45.85 \pm 1,29	24.72 \pm 2.48	33.62 \pm 2.36	37.88 \pm 0.54	47.54 \pm 1.36	56.42 \pm 2.20	54.67 \pm 2.24	49.13 \pm 1.32	37.33 \pm 3.01
Tri_16	24.46 \pm 2.18	2.45 \pm 1.22	33.38 \pm 2.56	43.61 \pm 5.35	44.82 \pm 0.75	28.89 \pm 4.60	37.67 \pm 0.89	42.54 \pm 2.01	31.79 \pm 27.77	36.35 \pm 31.79	43.94 \pm 39.36	38.66 \pm 24.18	37.83 \pm 6.32
Veg_87	46.04 \pm 0.64	1.90 \pm 0.98	26.64 \pm 1.52	26.57 \pm 3.37	60.07 \pm 3.85	51.14 \pm 9.56	41.11 \pm 2.78	39.15 \pm 3.82	39.78 \pm 2.84	45.90 \pm 1.54	52.30 \pm 1.48	44.28 \pm 2.31	33.04 \pm 1.74
Veg_17	41.28 \pm 1.11	2.39 \pm 1.89	40.48 \pm 4.34	42.19 \pm 4.73	45.38 \pm 0.53	47.30 \pm 3.54	43.84 \pm 2.19	51.22 \pm 1.31	46.27 \pm 2.04	43.82 \pm 2.39	46.30 \pm 1.12	46.90 \pm 1.70	45.04 \pm 1,54
Vol_84	54.17 \pm 0.96	2.76 \pm 0.51	35.85 \pm 1.22	46.54 \pm 2.91	46.81 \pm 3.44	28.29 \pm 2.62	39.37 \pm 0.52	47.13 \pm 4.90	51.92 \pm 3.92	60.81 \pm 0.84	61,31 \pm 1.96	55.29 \pm 2.66	45.36 \pm 1.17
Vol_85	53.38 \pm 0.70	8.33 \pm 4.27	42.13 \pm 1.24	46.47 \pm 3.64	52.58 \pm 6.47	41.34 \pm 5.68	45.63 \pm 1.85	45.92 \pm 6.08	50.55 \pm 3.87	60.35 \pm 1.21	61.19 \pm 2.99	54.50 \pm 3.49	46.27 \pm 2.23
Vol_86	53.01 \pm 1.97	4.00 \pm 2.01	52.38 \pm 3.56	58.97 \pm 3.43	49.79 \pm 3.18	37.21 \pm 2.36	49.56 \pm 2.56	60.47 \pm 2.40	56.24 \pm 0.58	59.06 \pm 1.02	63.49 \pm 3.45	59.81 \pm 1.30	57.01 \pm 1.14
Vou_16	76.86 \pm 6.62	42.24 \pm 28.37	47.16 \pm 5.92	50.53 \pm 8.44	47.47 \pm 0.84	44.31 \pm 6.90	47.37 \pm 2.26	59.14 \pm 15.70	55.35 \pm 6.56	58.18 \pm 1.41	57.15 \pm 2.14	57.45 \pm 4.71	53.05 \pm 8.86
Yli_90	81.61 \pm 2.28	18.66 \pm 7.68	51.21 \pm 2.44	53.27 \pm 5.02	62.65 \pm 1.50	49.12 \pm 15.52	54.06 \pm 3.62	60.26 \pm 2.84	57.06 \pm 5.04	58.19 \pm 3.69	59.48 \pm 4.00	58.75 \pm 3.13	55.45 \pm 2.62

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 61.53 ± 6.56 (Lake Lysimachia); 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 Kastoria 2016).

The linear regression of TSI_{ROT} and TSI_{CR} formulae with the eutrophication proxies revealed that TSI_{ROT} formulae TSI_{ROT1} and TSI_{ROT2} were correlated significantly with both TSI_{SD} (Appendix 5) and phytoplankton biomass (Table 4.3, Appendix 6)). On the other hand, the formulae TSI_{ROT3} and TSI_{ROT4} were not correlated significantly with either TSI_{SD} (Appendix 5) or phytoplankton biomass (Table 4.3, Appendix 6)). The TSI_{CR} formulae TSI_{CR1} , TSI_{CR2} and TSI_{CR3} were correlated significantly with both TSI_{SD} (Appendix 5) and phytoplankton biomass (Appendix 6), while TSI_{CR4} was correlated significantly with the TSI_{SD} but not with phytoplankton biomass (Table 4.3).

Table 4.3 Results of linear regression analysis for TSI_{ROT} formulae (TSI_{ROT1} - TSI_{ROT4}) and TSI_{CR} formulae (TSI_{CR1} - TSI_{CR4}) against TSI_{SD} and mean summer phytoplankton biomass (mg/L)

TSI_{SD}				Phytoplankton biomass (mg/L)				
Relationship	F	R ²	p	Relationship	F	R ²	P	
TSI_{ROT1}	$y = 16.91 + 0.45 x$	39.448	0.374	< 0.0001	$y = 38.93 + 0.64 x$	18.368	0.213	< 0.001
TSI_{ROT2}	$y = 23.77 + 0.42 x$	25.430	0.278	< 0.0001	$y = 44.26 + 0.62 x$	14.451	0.175	< 0.001
TSI_{ROT3}	$y = 46.05 + 0.08 x$	2.977	0.043	> 0.05	$y = 50.12 + 0.12 x$	2.018	0.029	> 0.05
TSI_{ROT4}	$y = 35.86 + 0.07 x$	0.981	0.015	> 0.05	$y = 39.84 + 0.03 x$	0.07	0.001	> 0.05
TSI_{CR1}	$y = 29.84 + 0.37 x$	36.223	0.354	< 0.0001	$y = 47.04 + 0.62 x$	29.296	0.301	< 0.0001
TSI_{CR2}	$y = 29.02 + 0.35 x$	21.164	0.243	< 0.0001	$y = 45.47 + 0.51 x$	12.81	0.159	< 0.001
TSI_{CR3}	$y = 36.85 + 0.28 x$	11.897	0.153	< 0.001	$y = 50.81 + 0.35 x$	4.923	0.068	< 0.05
TSI_{CR4}	$y = 45.31 + 0.19 x$	4.265	0.061	<0.05	$y = 54.92 + 0.22 x$	1.595	0.023	> 0.05

Based on these formulae that significantly correlated ($p < 0.001$) with both eutrophication proxies (Table 4.3), a new index was developed, 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). TSI_{CR3} was not included in the new index, since it was less significant correlated with TB ($p < 0.05$, $R^2 = 0.068$). The mean TSI_{ZOO} ranged from 33.04 ± 1.74 (Lake Vegoritis, 1987) to 58.65 ± 0.42 (Lake Doirani) (Table 4.2).

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

4. Trophic State Indices

$\text{TSI}_{\text{CR}1} - \text{TSI}_{\text{CR}4}$) and TSI_{zoo} (the average of $\text{TSI}_{\text{ROT}1}$, $\text{TSI}_{\text{ROT}2}$, $\text{TSI}_{\text{CR}1}$ and $\text{TSI}_{\text{CR}2}$), on the eutrophication proxies, TSI_{SD} and phytoplankton biomass (Table 4.4, Appendix 7). All indices were significantly correlated with both eutrophication proxies (Table 4.4). Based on Table 4.4 the best fitted index was TSI_{zoo} while none of the three zooplanktonic TSI indices were correlated with the percentage of cyanobacteria to total phytoplankton biomass (Table 4.5, Appendix 8).

Table 4.4 Results of linear regression analysis for TSI_{ROT} , TSI_{CR} and TSI_{zoo} against TSI_{SD} and phytoplankton biomass (mg/L)

	TSI _{SD}	Total phytoplankton biomass (mg/L)						
		Relationship	F	R ²	p	Relationship	F	R ²
TSI_{ROT}	$y = 30.65 + 0.26 x$	43.417	0.397	< 0.001	$y = 43.29 + 0.35 x$	18.186	0.211	< 0.0001
TSI_{CR}	$y = 35.26 + 0.30 x$	21.946	0.250	< 0.001	$y = 49.56 + 0.43 x$	12.05	0.151	< 0.001
TSI_{zoo}	$y = 24.31 + 0.42 x$	52.672	0.444	< 0.001	$y = 44.15 + 0.65 x$	30.873	0.312	< 0.0001

Table 4.5 Results of linear regression analysis for TSI_{ROT} , TSI_{CR} and TSI_{zoo} against the percentage contribution of cyanobacteria to total phytoplankton biomass

	Relationship	F	R ²	p
TSI_{ROT}	$y = 44.65 + 0.02 x$	0.663	0.014	> 0.05
TSI_{CRU}	$y = 44.65 + 0.02 x$	0.038	0.001	> 0.05
TSI_{zoo}	$y = 47.58 + 0.02 x$	0.248	0.005	> 0.05

The estimated trophic state based on (mean summer phytoplankton biomass, TSI_{SD} , TSI_{ROT} , TSI_{CR} and TSI_{zoo}) of each sampling was not the same in all cases (Appendix 9). However, when the mean values for each summer period were used for each zooplanktonic index (Table 4.6), there were cases identifying the same trophic state [i.e. Trichonis, Vegeritis (1987) and Volvi (1985)]. Still, the three zooplanktonic TSI indices (TSI_{ROT} , TSI_{CR} and TSI_{zoo}) only identified mesotrophic and eutrophic categories.

When TSI_{ROT} , TSI_{CR} and TSI_{zoo} were evaluated based on only the mean summer phytoplankton biomass, they detected different trophic categories (Table 4.6). Each index was significantly differentiated among the trophic state categories indicated by mean summer phytoplankton biomass (ANOVA, $p < 0.001$), however, the pairwise test revealed different potentials among the three indices to detect each trophic state. Different trophic states were detected by TSI_{ROT} (ANOVA, $F = 11,642$, $p < 0.0001$), TSI_{CR} (ANOVA, $F = 7.240$, $p < 0.001$) and TSI_{zoo} (ANOVA, $F = 13.200$, $p < 0.0001$). According to the post-hoc Bonferroni procedure oligotrophic lakes were differentiated from eutrophic and hypertrophic lakes by

all three indices (Bonferroni, $p < 0.05$), while, TSI_{CR} and TSI_{ZOO} did not discriminate oligotrophic from mesotrophic lakes (Figure 4.1)

Table 4.6 The trophic categories estimated for each lake using the mean values of different trophic state indices [TSI_{SD} , mean summer phytoplankton biomass (PB), TSI_{ROT} , TSI_{CR} and TSI_{ZOO}]. Codes abbreviation according to Table 4.2, m.d. missing data

Codes	TSI_{SD}	PB	TSI_{ROT}	TSI_{CR}	TSI_{ZOO}
Amv_16	Oligo	Oligo	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
Lys_16	Eu	Meso	Meso-Eu	Eu	Eu
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	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

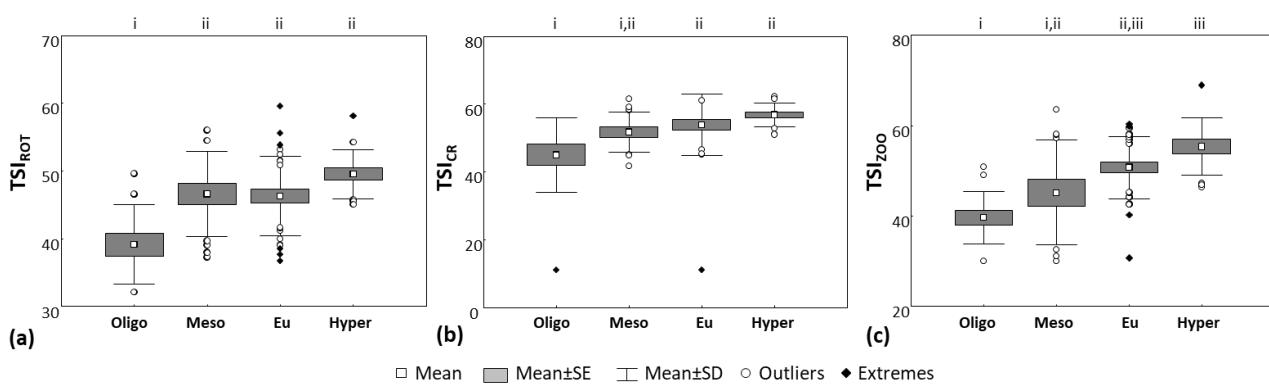


Figure 4.1 Box plots of (a) TSI_{ROT} , (b) TSI_{CR} and (c) TSI_{ZOO} grouped by trophic state (indicated by mean summer phytoplankton biomass). i, ii, iii Significant differences [Bonferroni procedure applied in cases with $p < 0.05$ (ANOVA)]

Based on the above results, especially those of Bonferroni procedure, a classification of only two trophic states –low (oligotrophic and mesotrophic lakes) and high (eutrophic and hypertrophic lakes) – might be more appropriate. The boundaries to detect low and high trophic state for TSI_{ROT} and TSI_{ZOO} should be set at 45 and for TSI_{CR} at 50 (Figure 4.2). Using these boundaries, the three zooplanktonic indices identified the same trophic category for 70% of the cases (Table 4.7). More specifically, the mean values of the summer period of TSI_{ROT} , TSI_{CR} and TSI_{ZOO} could detect correctly 67%, 79% and 83%, respectively, of the trophic state according to phytoplankton biomass, while according to TSI_{SD} 83% of the cases were detected correctly using all three zooplanktonic indices (Table 4.7). Moreover, all three zooplanktonic indices could detect the two trophic categories (low and high) as defined by mean summer phytoplankton biomass (t -test TSI_{ROT} : $p < 0.01$, TSI_{CR} : $p < 0.01$ and TSI_{ZOO} : $p < 0.001$) (Figure 4.2).

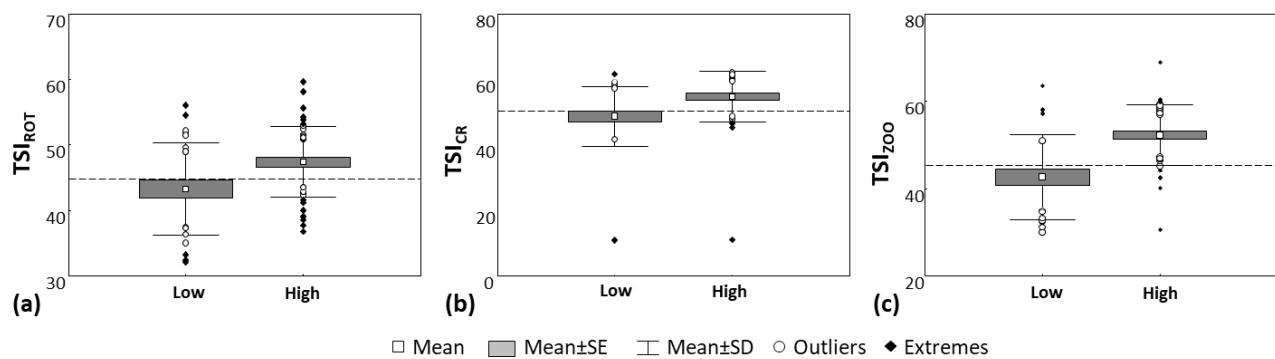


Figure 4.2 Box plots of (a) TSI_{ROT} , (b) TSI_{CR} and (c) TSI_{ZOO} grouped by low (oligotrophic–mesotrophic) and high (eutrophic–hypertrophic) trophic state (indicated by mean summer phytoplankton biomass). Dashed line indicates the boundary proposed in the present study for each index

4.5 Discussion

Trophic state indices based on zooplankton communities, TSI_{ROT} and TSI_{CR} , have recently been developed for Polish lakes (Ejsmont-Karabin 2012, Ejsmont-Karabin and Karabin 2013). TSI_{ROT} has been mainly applied in several studies in Poland (e.g. Gutkowska et al. 2013, Jekatierynczuk-Rudczyk et al. 2014, Dembowska et al. 2015, Marszelewski et al. 2017, Dunalska et al. 2018, Rosińska et al. 2019). Outside Poland, it has been used in China (Liang et al. 2019, Tang et al. 2019) and only TSI_{ROT1} in Mexico (Gutiérrez et al. 2017, Moreno-Gutiérrez et al. 2018) and Nigeria (Bolawa et al. 2018), while both TSI_{ROT1} and TSI_{ROT4} have

been used in the Estonian Lake Vortsjärv (Haberman and Haldna 2014). As for TSI_{CR} it has been mainly applied in Poland [as originally described (Ochocka and Pasztaleniec 2016, Dunalska et al. 2018) and only the formulae TSI_{CR3}, TSI_{CR4} and TSI_{CR6} (Jekatierynczuk-Rudczyk et al. 2014)] and in Nigeria (TSI_{CR5}) (Bolawa et al. 2018). In the Mediterranean zone TSI_{ROT1} has been applied in Portugal (Geraldes and Pasupuleti 2016) and TSI_{ROT1}, TSI_{CR1} and TSI_{CR2} in Spain (Álvarez-Manzaneda et al. 2019). The present study is the first application and evaluation of both TSI_{ROT} and TSI_{CR} for Greek lakes.

Table 4.7 The trophic categories estimated for each lake using the mean values of TSI_{SD} and summer phytoplankton biomass (PB) 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 abbreviation according to Table 4.2, m.d. missing data

Codes	TSI _{SD}	PB	TSI _{ROT}	TSI _{CR}	TSI _{ZOO}
Amv_16	Oligo	Oligo ¹	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
Lys_16	Eu	Meso	High	High	High
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 ²	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

In the present study TSI_{ROT} and TSI_{CR} was calculated as the average of 4 out of the 6 proposed formulae for each index due to either data limitations (for TSI_{ROT5}) or differences in the trophic state indicator species (for TSI_{ROT6} and TSI_{CR6}). Even though TSI_{ROT5} was not used due to data limitations, the percentage of *tecta* form in the *K. cochlearis* population might be

suitable for eutrophication assessment, as in the case of the Neva Estuary (Baltic Sea) (Gopko and Telesh 2013). Further analysis is needed in order to conclude if this ratio is suitable for the Mediterranean lakes. The TSI_{ROT6} and TSI_{CR6} formulae of the indicator species are also considered suitable metrics for eutrophication and ecological water quality assessment in Poland (Ochocka and Pasztaleniec 2016). However, different patterns of dominance for the indicator species for each region are recorded throughout the literature; [e.g. genus *Trichocerca* is an indicator of oligotrophic systems (Sládecèk 1983) while *Trichocerca capucina* and *Trichocerca pusilla* 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 conditions (Pejler 1983)]. This highlights the need of further research in order for TSI_{ROT6} and TSI_{CR6} to be adapted using indicator species of the studied region in general, in our case for the Mediterranean region.

The TSI_{ROT} and TSI_{CR} formulae were correlated with the two eutrophication proxies, phytoplankton biomass and TSI_{SD} , in the attempt to find the more efficient formulae for the assessment of Mediterranean systems. Acknowledging that the partial use of the formulae can be effective in the Mediterranean region (Geraldes and Pasupuleti 2016, Álvarez-Manzaneda et al. 2019), an index was developed as the average of only the formulae significantly correlated ($p < 0.001$) (TSI_{ROT1} , TSI_{ROT2} , TSI_{CR1} and TSI_{CR2}) with both eutrophication proxies. Thus, the new index namely TSI_{zoo} (Zooplankton Trophic State Index), can be used for the assessment of eutrophication based on the whole zooplankton community. According to the correlation of the three zooplanktonic indices (TSI_{ROT} , TSI_{CR} and TSI_{zoo}) with the two eutrophication proxies, TSI_{zoo} was the best fitted index and thus the most efficient in discriminating eutrophication.

In order to evaluate the zooplanktonic trophic state indices, the trophic state of the same lake/sampling was estimated using all trophic indices. Different trophic category was estimated for many cases when all indices or when only the zooplanktonic indices were used. Although such discordances among different trophic indices are expected both in reservoirs and natural lakes according to indices' characteristics (e.g. Duggan et al. 2001, García-Chicote et al. 2018) when the mean values of the summer period were used the differences were smoothed. For example, in the case of Lake Volvi in 1985 the trophic state varied from mesotrophic to eutrophic using monthly values of all three zooplanktonic TSI indices, while it was only meso-eutrophic when mean values were used. Hence, the assessment of the trophic

state should be done based on the mean summer values when the TSI_{ROT} , TSI_{CR} and TSI_{ZOO} are used.

Phytoplankton is the most reliable element for assessing trophic state (e.g. Lyche-Solheim et al. 2013, Katsiapi et al. 2016b). However, only Dembowska et al. (2015) correlated TSI_{ROT} with phytoplankton indices, while the rest of the studies applying TSI_{ROT} and TSI_{CR} indices/formulae used Carlson TSI indices or even other indices based on zooplankton in order to evaluate them (e.g. Jekatierynczuk-Rudczyk et al. 2014, Gutiérrez et al. 2017). TSI_{SD} is efficient and cost-effective for large scale and long-term monitoring when combined with low-frequency high quality phytoplankton data during the warm period for the assessment of Mediterranean lakes (Katsiapi et al. 2016b). However, in our case TSI_{SD} overestimated the trophic state of Lake Ozeros, characterized by increased detritus, the deep lakes Trichonis and Vegeritis [suffering from a continuous deficit in water balance and drastic water level fluctuations, which might affect the TSI_{SD} (Katsiapi et al. 2016b)] as well as the reservoirs (Tavropos and Kremasta). Reservoirs as riverine systems are characterized by increased organic material (total suspended solids or allochthonous dissolved organic matter) lowering transparency (e.g. Mash et al. 2004, Bolgrien et al. 2009). Based on these differentiations, it is evident that TSI_{SD} 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 the most accurate eutrophication proxy.

Evaluating the zooplanktonic TSI indices based on the proposed boundaries (Ejsmont-Karabin 2012, Ejsmont-Karabin and Karabin 2013) it is evident that these boundaries should be revised for the Mediterranean region. Oligotrophic lakes were overestimated since there is no proposed boundary for them; all lakes with $TSI < 45$ were classified as mesotrophic. Hypertrophic lakes were underestimated since none of the three indices took values over 65. Furthermore, TSI_{ROT} had lower values compared to TSI_{CR} leading in the misclassification of the trophic state for many cases and in the underestimation of the majority of the studied eutrophic lakes. This was expected since crustacean communities are influenced by both top-down and bottom-up forces reflecting also the impact of fish predation (Ejsmont-Karabin and Karabin 2013) which is more increased in the Mediterranean zone (Beklioglu et al. 2007, Moustaka-Gouni et al. 2014). However, TSI_{CR} had very low values in the samplings of July of lakes Trichonis and Ozeros because cyclopoid copepods were not recorded in the crustacean's abundance resulting in zero values for the TSI_{CR2} , TSI_{CR3} and TSI_{CR4} formulae. This indicates

that, TSI_{CR} should not be used in lakes without cyclopoids or when no cyclopoids are recorded. TSI_{zoo} incorporating formulae of both indices was more accurate in estimating the trophic state, strengthening the result of being the most efficient of the zooplankton TSI indices in discriminating eutrophication.

Estimations of different trophic state using TSI_{ROT} or TSI_{CR} and other trophic indices, have also been recorded elsewhere e.g. in Mexico (Moreno-Gutiérrez et al. 2018) and even in Polish lakes (e.g. Jekatierynczuk-Rudczyk et al. 2014). Cyanobacterial blooms have been proposed as a possible reason for the misestimation of the eutrophic and hypertrophic lakes using TSI_{ROT} (Dembowska et al. 2015). It is well known that Cyanobacteria affect the zooplankton community reducing their feeding rates (e.g. malfunction of the filtering limbs in cladocerans) and combined with their poor nutritional quality lead to lower development of body size, slower reproduction and higher mortality (Lampert et al. 1987, Ger et al. 2016). This effect might be more intense in Mediterranean lakes, since prolonged cyanobacterial blooms (up to 8 months) have been recorded with more than 90% cyanobacterial contribution to the phytoplankton community of eutrophic lakes (Vardaka et al. 2005). This is also evident in the studied lakes, with cyanobacteria recorded in all lakes except from Kremasta Reservoir, and being 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, 99.96% in the deep Lake Trichonis and 100% in the hypertrophic Lake Pamvotis (Appendix 4)]. Nevertheless, no statistically significant relationship was recorded between the zooplanktonic TSI indices and the percentage contribution of the cyanobacteria biomass in the studied lakes.

Despite the discrepancies recorded when the estimated trophic state of each lake/sampling was compared, all zooplanktonic indices (TSI_{ROT} , TSI_{CR} , TSI_{zoo}) were significantly differentiated among the groups of trophic state indicated by mean summer phytoplankton biomass. The differentiation of oligotrophic lakes using the TSI_{ROT} is important because the dataset used for TSI_{ROT} development did not include oligotrophic lakes (Ejsmont-Karabin 2012), indicating that oligotrophic lakes might be differentiated from mesotrophic even in temperate zone. Thus, further research is needed in order to test this differentiation and to propose a boundary between oligotrophic and mesotrophic lakes for temperate lakes. TSI_{CR} and TSI_{zoo} differentiated significantly the oligotrophic category from the eutrophic and hypertrophic categories but did not differentiate oligotrophic from mesotrophic lakes. Although, according to these results the need to modify the proposed boundaries is evident,

values overlaps do not allow that. However, zooplanktonic indices could be used to differentiate two trophic groups: the low trophic state (including oligotrophic and mesotrophic categories), and the high trophic state (including eutrophic and hypertrophic categories). The boundaries for discriminating the groups of low and high trophic state for TSI_{ROT} and TSI_{ZOO} could remain at $TSI = 45$, as already proposed by Ejsmont-Karabin (2012) but for TSI_{CR} the boundary should be set a bit higher at $TSI_{CR} < 50$. Applying the new boundaries TSI_{ZOO} classified correctly most of the studied lakes, except for four cases. Lake Trichonis was underestimated possibly due to the increased fish predation by *Atherina boyeri* (Chrisafi et al. 2007). Lake Lysimachia, even though a mesotrophic lake, had a domination of cyanobacteria >99% in July and September and Lake Paralimni had increased rotifers abundance and domination (> 70%) increasing the TSI_{ROT1} and TSI_{ROT2} formulae. Lake Ozeros had increased abundance of detritus indicating the need for further investigation on the trophic state.

4.6 Conclusions

In conclusion, both the Rotifer Trophic State Index (TSI_{ROT}) and the Crustacean Trophic State Index (TSI_{CR}) applied on data from the zooplankton communities of 17 Greek lakes were increasing across the eutrophication gradient in Mediterranean region. Furthermore, a new index namely the Zooplankton Trophic State Index TSI_{ZOO} was developed as the average of the significantly correlated formulae with the eutrophication proxies (TSI_{SD} and phytoplankton biomass) combining partially both TSI_{ROT} and TSI_{CR} . The evaluation of the indices revealed that they can detect efficiently two groups of low (oligotrophic-mesotrophic) and high (eutrophic-hypertrophic) trophic state when applied in the Mediterranean region. In addition, the boundary for TSI_{ROT} and TSI_{ZOO} was set to be < 45 for low category and was modified for TSI_{CR} as <50 for Mediterranean lakes. Overall, TSI_{ROT} and TSI_{CR} can be promising and useful tools for trophic state assessment since they need low level identification skills (do not require species-level identifications) and zooplankton has easy and inexpensive samplings. It should be taken into consideration that TSI_{CR} should not be used in lakes without cyclopoid copepods. TSI_{ZOO} was found to be the most efficient of the three zooplankton indices, since it was the one with better correlation to eutrophication and estimations of the trophic state especially when the mean summer value was used. Thus, it could be considered as a parameter of a multi-metric index of ecological water quality, reflecting eutrophication pressure based on zooplankton

4. Trophic State Indices

and TSI_{zoo} is proposed as a promising, effective and cost-effective tool for large scale and long-term monitoring programs of Mediterranean lakes.



5. Grazing Potential

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5.1 Abstract

Grazing Potential (GP, in %/day) is an index measuring the potential top-down control of phytoplankton by zooplankton, showing the percentage of the phytoplankton biomass ingested per day. In the present study, it was estimated based on data from plankton communities of 13 Greek natural lakes covering the entire trophic spectrum, in order to test its efficiency in discriminating classes of ecological water quality. Lakes with high GP values were characterized by increased biomass dominated by large-bodied zooplankton [cladocerans (e.g. *Daphnia*, *Diaphanosoma*) or/and calanoid copepods] while lakes with low GP values exhibited increased phytoplankton biomass and/or domination of small-bodied zooplankton indicating intensive fish predation. GP successfully discriminated among ecological water quality classes (ranged from poor to high) as estimated using a phytoplankton-based water quality index, the PhyCol Index. Moreover, it was significantly correlated with TSI_{SD}, an index of eutrophication based on secchi depth. Thus, GP is a promising and effective metric for ecological water quality assessment. As a next step, PhyCol index was modified to include GP as a metric to enhance the phytoplankton-based ecological status assessment incorporating zooplankton as a supporting factor. The modified index, namely PhyCol_{GP}, consisted of six metrics of composition and functional structure of plankton communities. It successfully assessed the ecological water quality in accordance with PhyCol classification whereas it was also significantly correlated with TSI_{SD}. Hence, the modified index PhyCol_{GP} is recommended as a robust index since it can reflect various responses to environmental change and should be used for monitoring the ecological water quality of Mediterranean lakes.

Περίληψη

Το Δυναμικό Βόσκησης (GP, σε %/μέρα) είναι ένας δείκτης που μετρά το δυνητικό έλεγχο του φυτοπλαγκτού από το ζωοπλαγκτό μέσω του ελέγχου από την κορυφή προς τη βάση, και δείχνει το ποσοστό της φυτοπλαγκτικής βιομάζας που μπορεί να καταναλωθεί ημερησίως. Στην παρούσα έρευνα, εκτιμήθηκε με βάση δεδομένα των πλαγκτικών κοινωνιών από 13 φυσικές ελληνικές λίμνες που καλύπτουν όλο το εύρος της τροφικής κατάστασης, με σκοπό να διερευνηθεί η αποτελεσματικότητά του στο να διακρίνει τις

κατηγορίες οικολογικής ποιότητας των υδάτων. Λίμνες με υψηλές τιμές GP χαρακτηρίζονται από υψηλές τιμές βιομάζας που κυριαρχούνταν από μεγαλόσωμο ζωοπλαγκτό [κλαδοκερωτά (π.χ. *Daphnia*, *Diaphanosoma*) ή/και καλανοειδή κωπήποδα], ενώ λίμνες με χαμηλές τιμές GP παρουσίασαν αυξημένη βιομάζα φυτοπλαγκτού και/ή κυριαρχία μικρόσωμου ζωοπλαγκτού που αποτελεί ένδεικη έντονης θήρευσης από τα ψάρια. Το GP διέκρινε με επιτυχία τις κατηγορίες οικολογικής ποιότητας υδάτων (οι οποίες κυμάνθηκαν από ελλιπής έως υψηλή), όπως εκτιμήθηκαν από τον δείκτη ποιότητας υδάτων PhyCol που βασίζεται στο φυτοπλαγκτό. Επιπλέον συσχετίστηκε σημαντικά με το TSI_{SD}, ένας δείκτης ευτροφισμού με βάση τη διαφάνεια δίσκου secchi. Φαίνεται λοιπόν ότι, το GP είναι μια πολλά υποσχόμενη και αποτελεσματική μετρική για την εκτίμηση της οικολογικής ποιότητας των υδάτων. Σε ένα επόμενο βήμα, ο δείκτης PhyCol τροποποιήθηκε για να συμπεριλάβει το GP ως μετρική για να ενισχυθεί η ταξινόμηση της οικολογικής κατάστασης των λιμνών με βάση το φυτοπλαγκτό, ενσωματώνοντας το ζωοπλαγκτό ως παράγοντα υποστήριξης. Ο τροποποιημένος δείκτης, που ονομάστηκε PhyCol_{GP}, αποτελείται από έξι μετρικές με βάση τη σύνθεση και τη λειτουργική δομή των πλαγκτικών κοινωνιών. Αξιολόγησε με επιτυχία την οικολογική ποιότητα των υδάτων σε συμφωνία με την ταξινόμηση του PhyCol, ενώ συσχετίστηκε σημαντικά με το TSI_{SD}. Ως εκ τούτου, ο τροποποιημένος δείκτης PhyCol_{GP} προτείνεται ως ένας ισχυρός δείκτης δεδομένου ότι αντικατοπτρίζει διαφορετικές αποκρίσεις σε περιβαλλοντικές αλλαγές και πρέπει να χρησιμοποιείται για την παρακολούθηση της οικολογικής ποιότητας των υδάτων των μεσογειακών λιμνών.

5.2 Introduction

Zooplankton grazing on phytoplankton is one of the central parts of the pelagic food web function as being the main step of the top-down trophic cascade control and the central force in biomanipulation restoration (Carpenter et al. 1985, Sommer 2008). The main zooplankton players in this are the efficient filter feeders of the genus *Daphnia* and the calanoid copepods (e.g. Sommer and Stibor 2002, Hessen and Kaartvedt 2014). Daphnids as keystone species are able to effectively control the available phytoplankton biomass, feeding mainly on pico- and nanophytoplankton up to 35 µm (Gophen and Geller 1984), leading to the annual clear water phase (Lampert et al. 1986). This can be further supported by calanoid copepods which are also herbivorous feeding mainly on larger algae (De Mott and Watson

1991, Dussart and Defaye 2001) and less by rotifers being suspension feeders feeding within the food size spectrum of daphnids (Wallace et al. 2002, Sommer and Sommer 2006).

Of major importance for the effective grazing effect is the suitability of phytoplankton as food (Arp and Deneke 2007). Two main factors seem to play an important role in determining phytoplankton suitability as food: the biochemical properties of the different species, or even different strains of the same species; and the shape and size of the colonies (de Bernardi and Giussani 1990). For example, cryptophytes, chlorophytes, chrysophytes, and diatoms had adequate concentrations of biomolecules (such us phosphorus, ω-3 FAs and sterols) being important for zooplankton growth and reproduction (e.g. Taipale et al. 2013, Peltomaa et al. 2017) while dinoflagellates are considered as a poor food due to their size and spiny shape and even if they are a suitable food for some cyclopoids they are not a preferred food source (Santer 1996). Moreover, it is well established that cyanobacteria affect the zooplankton community due to having poor nutritional quality, producing toxins and large colonies leading to the malfunction of the filtering limbs of cladocerans (e.g. Lampert 1987, de Bernardi and Giussani 1990, Ger et al. 2016).

Grazing Potential (GP, in %/day) is an index developed based on data of Danish lakes and it is basically a measure of the potential top-down control of phytoplankton by zooplankton, showing the percentage of the phytoplankton biomass ingested per day (Jeppesen et al. 1997). In the original description of GP, only crustacean biomass was used assuming that cladocerans and copepods ingest phytoplankton corresponding to 100% and 50%, respectively, of their biomass per day; copepods especially cyclopoids are not only herbivorous eating algae and diatoms but also carnivorous (Brandl 1998, Fryer 2009). Acknowledging the theory of the functional characteristics of plankton communities, such as the size and the shape of phytoplankton which affect their susceptibility and edibility (Weithoff 2003) and the feeding strategies of zooplankton groups (Barnett et al. 2007) GP was later modified twice based on data from German lakes. First by Arp and Deneke (2007) with phytoplankton taxonomic groups getting weighting factors in order to estimate only the edible phytoplankton biomass and finally, by Arp et al. (2010) with the addition of rotifers biomass in order for the whole zooplankton community to be represented.

GP was developed and modified based on data from temperate lakes, showing seasonal variation with low values during summer which tend to decrease across the eutrophication gradient (Jeppesen et al. 1997). However, since Mediterranean lakes are

differentiated from temperate lakes altering the patterns described for plankton communities of the well-studied cold-temperate European lakes as in the case of the PEG model (Moustaka-Gouni et al. 2014) the aim of the present study was to explore the patterns of GP during the summer period in a different climatic zone, in this case the Mediterranean region, and to examine the sensitiveness of the GP in discriminating different categories of ecological water quality indicating its potential use as a metric for ecological water quality assessment. For these, the variability of GP was examined using data from summer plankton communities from 13 natural Greek lakes, along the entire trophic spectrum and a wide range of lakes' morphometry and water transparency. To test that GP's variability could differentiate ecological water quality, PhyCol index (Katsiapi et al. 2016b), an ecologically sound index developed using phytoplankton data from Greek lakes, was used to estimate the ecological water quality classes and then GP was tested in discriminating the above ecological quality classes. As a next step, GP was incorporated as a metric in a modified PhyCol index, namely PhyCol_{GP}, hypothesizing that this more functional phytoplankton community index incorporating zooplankton as a supporting factor, will improve the assessment of lake ecological water quality compared to PhyCol. In order to test this the PhyCol_{GP} and PhyCol application in Greek lakes and their relation to the eutrophication proxy TSIs_{SD} were tested.

5.3 Material & Methods

Grazing Potential

GP was applied on data from the plankton communities of 13 natural Greek lakes of different altitude, surface area, depth and trophic state (Figure 2.1, Table 2.1). Not all data of the studied lakes (as described in Chapter 2) were used, since lakes Kremasta and Tavropos are reservoirs, Lake Ozeros was dominated by increased mollusc larvae (not included in the index), and the cases of Lake Yliki and lakes Vegeritis (1987) and Kastoria (1999) due to phytoplankton data limitation.

The modified equation (14) of GP (in % /day) was calculated according to Arp et al (2010) based on the dry biomass of zooplankton and phytoplankton communities having weighted values for the various groups (Jeppesen et al. 1997, Arp and Deneke 2007, Arp et al 2010). The weighted phytoplankton groups comprised the edible phytoplankton biomass (B_{ED}) according to Equation (15).

$$GP = \frac{B_{ROT} + B_{CLAD} + 0.5 B_{COP}}{B_{ED}} \quad (14)$$

$$B_{ED} = 0.3 B_{CYANO} + 0.5 B_{CHLORO} + 0.5 B_{CHRYSO} + 1 B_{CRYPTO} + 1 B_{PRYMNESIO} + 0.7 B_{DIATOMS} + 0 B_{DINO} + \\ 0.3 B_{CONJ} + 0.5 B_{EUGLENO} \quad (15)$$

Where: B is the dry biomass (mg/L) of rotifers (ROT), cladocerans (CLAD), copepods (COP), cyanobacteria (CYANO), chlorophytes (CHLORO), chrysophytes (CHRYSO), cryptophytes (CRYPTO), prymnesiophytes (PRYMNESIO), diatoms (DIATOMS), dinophytes (DINO), conjugatophytes (CONJ) and euglenophytes (EUGLENO). The phytoplankton groups were weighted according to their relative edibility ranging from very good (1) to not at all (0) edible.

The PhyCol_{GP} Index

The PhyCol_{GP} index is a modified PhyCol index incorporating GP as a metric, thus it is a phytoplankton community index incorporating zooplankton as a supporting factor including additional information on the lake plankton community for ecological water quality assessments. In particular, PhyCol_{GP} includes the five metrics/sub-indices [total phytoplankton biovolume (TB), cyanobacterial biovolume according to WHO (World Health Organization) Guidelines (WG), modified Nygaard sub-index based on the biomass of indicator taxonomic groups (NB), modified Nygaard sub-index based on species richness of indicator taxonomic groups (NS), Quality Group species Index (QG)] originally included in PhyCol and an additional sixth metric, the GP. It is calculated as the sum of the scores of the six metrics/sub-indices (Table 5.1): PhyCol_{GP} = TB + WG + NB + NS + QG + GP.

PhyCol_{GP} ranges from zero to five representing five classes of ecological quality: 0–1: Bad, >1–2: Poor, >2–3: Moderate, >3–4: Good, >4–5: High/reference.

According to PhyCol methodology, each metric has a classification scheme of 5 categories, each category having a different score. In order for PhyCol_{GP} to follow PhyCol values range and the same boundaries for ecological water quality assessment, the scores of the five metrics in PhyCol were re-assigned in order to include in the same total score of PhyCol the 6th metric of GP. Following PhyCol methodology, the selected scoring system for PhyCol_{GP} also gives a specific weight to the different metrics/sub-indices according to their importance on detecting water quality changes (Katsiapi et al. 2016b). Since GP is a food web metric combining the functional trait characteristics of both zooplankton and phytoplankton communities, it was given 0.8 as a score in the first (reference) category, which is comparable to the corresponding ones of the rest of the metrics/sub-indices (0.5 to 0.8) except for the

higher score (1.8) of the metric TB. The PhyCol_{GP} score for WG, NB, NS and QG metrics/sub-indices were based on their calculation and the classification schemes as they are described in Katsiapi et al. (2016b) and for TB as it is described in Stamou et al. (2019b). In order to set the boundaries of the GP metric for the five quality classes, the mean GP values of each ecological water quality class (estimated by PhyCol) was used as the lower boundary for each class of ecological water quality.

Water Quality Assessment

The assessment of the ecological water quality was made using the phytoplankton PhyCol index according to Katsiapi et al. (2016b) and Stamou et al. (2019b). Linear Regression was applied to test the dependence of GP on ecological water quality as assessed by PhyCol and eutrophication based on TSI_{SD}. Phytoplankton biomass even though the most appropriate eutrophication proxy was not used since it is the main metric of PhyCol. Moreover, in order to evaluate the application of GP in assessing ecological water quality, we used the ecological water quality classification based on PhyCol (Table 2.1). ANOVA and Bonferroni procedure were applied to reveal if the GP differed among the four groups (no lake is classified as “bad”) of ecological water quality. Weight cases for each parameter were used to reduce bias due to there being different number of lakes or samplings in each class.

5.4 Results

Grazing Potential across Lake Trophic Spectrum and Ecological Water Quality

Grazing potential (GP) applied on the plankton communities of the 13 Greek studied lakes ranged from 0.18% /day (Lake Voulkaria in July 2016) to 146.55% /day (Lake Kastoria in July 2016) (Figure 5.1). GP did not show a specific pattern along the trophic spectrum.

Table 5.1 Metrics included in the PhyCol_{GP} Index, their classification scheme and respective scores for its calculation.

Metrics/Sub-Indices		Scores				
Total Phytoplankton Biovolume (TB)	Classification scheme ¹	Reference or Ultraoligo/Oligotrophic	Good or Mesotrophic	Moderate or Eutrophic I	Poor or Eutrophic II	Bad or Hypertrophic
	PhyCol _{GP} Score	1.8	1.4	0.8	0.4	0.2
WHO Guidelines (WG)	Classification scheme	<Level 1	Level 1: 0.2–1 mm ³ /L	Level 1: >1 mm ³ /L	Level 2: >10 mm ³ /L	Scum formation
	PhyCol _{GP} Score	0.8	0.7	0.4	0.3	0.1
Nygaard Biomass (NB)	Classification scheme	0–1	1–10	10–100	100–1000	>1000
	PhyCol _{GP} Score	0.5	0.4	0.3	0.2	0.1
Nygaard Species (NS)	Classification scheme	<2	2–4	>4–6	>6–8	>8
	PhyCol _{GP} Score	0.6	0.5	0.3	0.2	0.1
Quality Group species sub-Index (QG)	Classification scheme	>60	46–60	31–45	16–30	<16
	PhyCol _{GP} Score	0.5	0.4	0.3	0.2	0.1
Grazing Potential (GP)	Classification scheme	>120	55–120	15–55	5–15	<5
	PhyCol _{GP} Score	0.8	0.7	0.4	0.3	0.1

¹ The TB classification scheme is developed in Stamou et al. (2019b)

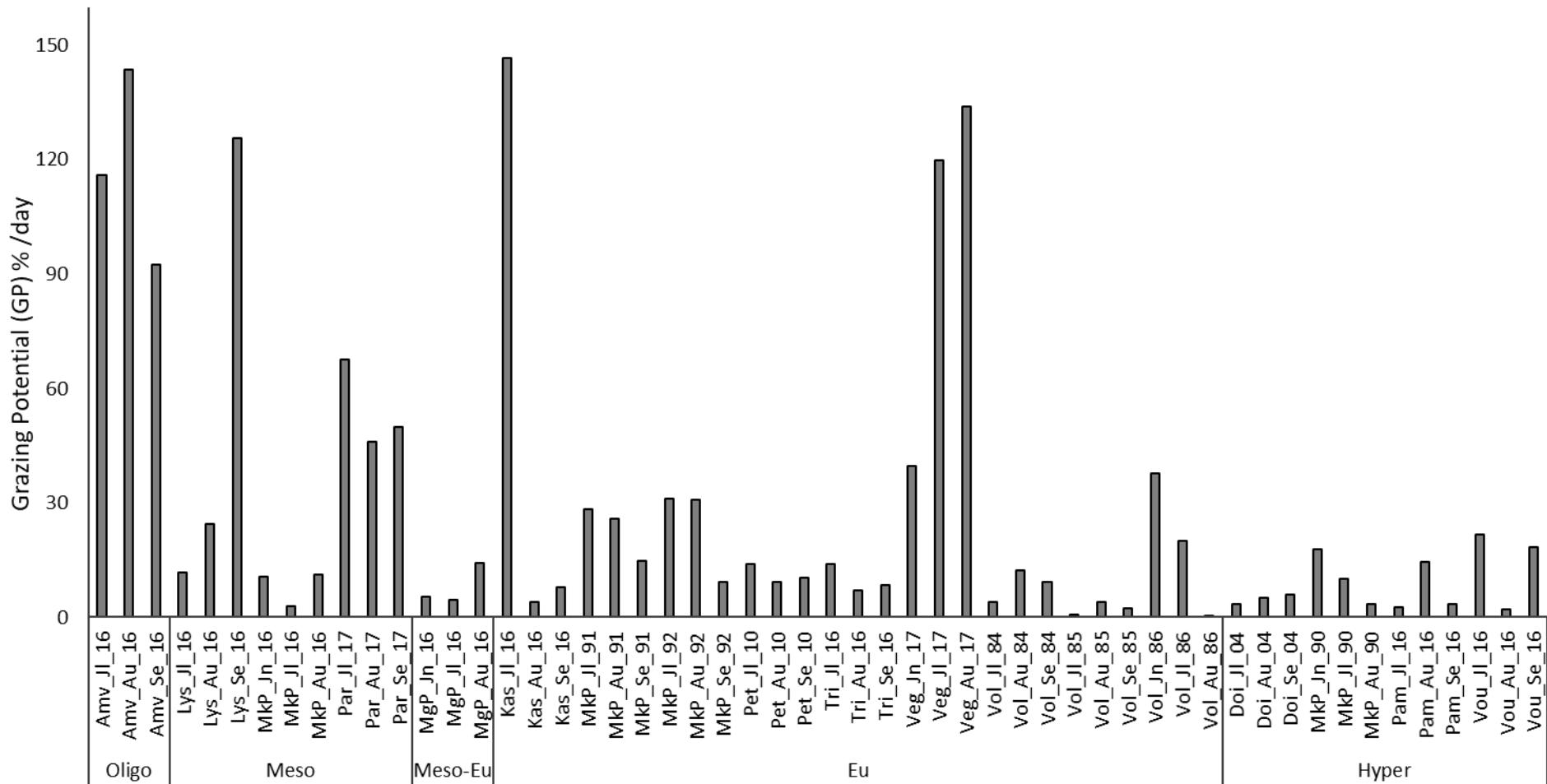


Figure 5.1 Grazing potential values of the 13 Greek lakes in the various samplings (abbreviations according to Figure 3.1) across the trophic spectrum

GP was significantly correlated with PhyCol ($GP = 28.40 \text{ PhyCol} - 46.71$; $R^2 = 0.40$ and $p < 0.0001$) and TSI_{SD} ($GP = 81.45 - 0.94 \text{ TSI}_{SD}$; $R^2 = 0.13$ and $p = 0.008$) (Figure 5.2). GP successfully detected different classes of ecological water quality as indicated by PhyCol (ANOVA: $F = 305.139$, $p < 0.0001$) while the pairwise test also indicated significant differences among the four categories (Bonferroni: $p < 0.0001$).

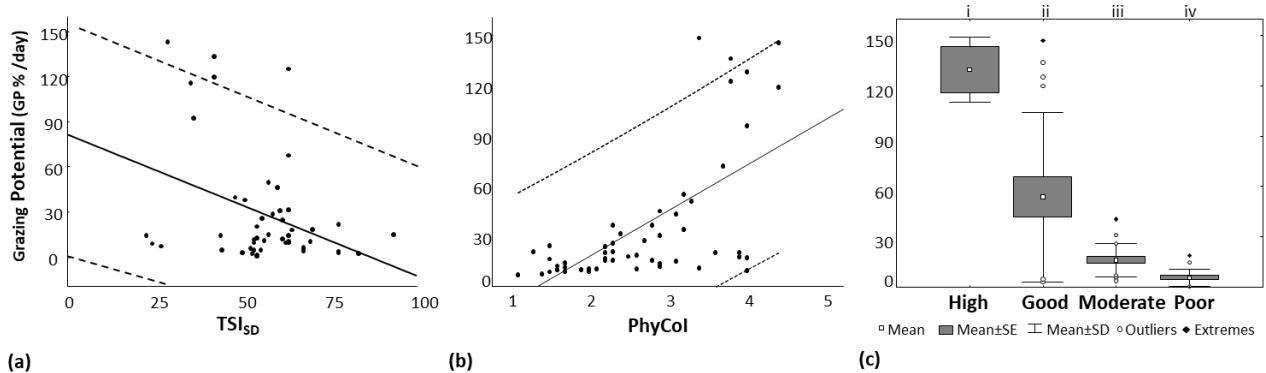


Figure 5.2 Scatter plot of grazing potential (GP) against (a) TSI_{SD} and (b) PhyCol, solid line indicates the linear regression line and dashed lines indicate 95% prediction limits of the model; (c) box plot of GP from the 13 Greek lakes grouped into ecological water quality classes according to PhyCol. i, ii, iii, iv indicate significant differences [Bonferroni procedure applied in cases with $p < 0.05$ (ANOVA)]

However, range overlaps were recorded among the different classes and almost 28% of the cases were extremes and outliers. In detail, the extreme values were Lake Kastoria (July 2016) for good class, Lake Vegoritis (June 2017) for the moderate class and Lake Voulkaria (September 2016) for the poor class. The outliers were indicating either better or lower ecological water quality. Outliers with high value were lakes Vegoritis (July and August 2017) and Lysimachia (September 2016) for the good class, Lake Mikri Prespa (July and August 1992) for the moderate class and Lake Pamvotis (August 2016) for the poor class. Outliers with low value were lakes Mikri Prespa and Megali Prespa (July 2016) for the good class, lakes Kastoria (August 2016), Volvi (July 1984) and Megali Prespa (June 2016) for the moderate class and Lake Voulkaria (July 2016) for poor class (Figure 5.2).

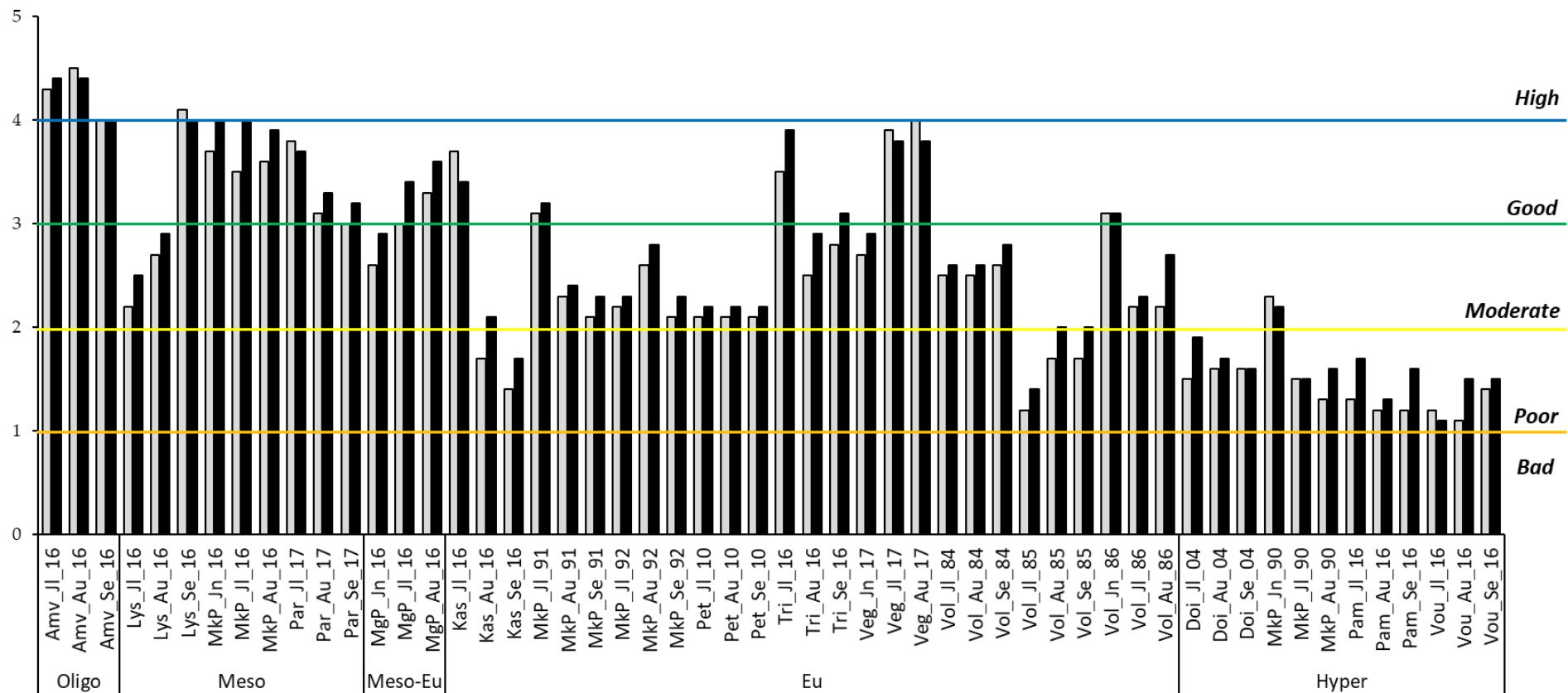


Figure 5.3 *PhyCol* (black) and *PhyCol_{GP}* (grey) values for the 13 Greek lakes in the various sampling dates (abbreviations according to Figure 3.1) across the trophic spectrum

PhyCol_{GP} Application Versus PhyCol

Both PhyCol and PhyCol_{GP} indices were used to assess the ecological water quality of the 13 Greek lakes and their values are presented in Figure 5.3. The PhyCol index ranged from 1.1 (Lake Voulkaria in July 2016) to 4.4 (Lake Amvrakia in July and August 2016).

The PhyCol_{GP} index ranged from 1.1 (Lake Voulkaria in August 2016) to 4.5 (Lake Amvrakia in August 2016). Both PhyCol and PhyCol_{GP} classified the ecological water quality of the 13 lakes in the same category except for the cases of Lake Kastoria in August 2016, Lake Lysimachia in September 2016, Lake Megali Prespa in July 2016, Lake Paralimni in September 2017 and Lake Trichonis in September 2016. Both PhyCol and PhyCol_{GP} values exhibited great variability within the same lake (Figure 5.3).

Based on the dataset of the studied lakes, the linear regression analysis revealed that both PhyCol_{GP} and PhyCol had a significant negative relationship with the eutrophication proxy TSI_{SD} index ($\text{PhyCol}_{\text{GP}} = 5 - 0.04 \text{TSI}_{\text{SD}}$; $R^2 = 0.46$ and $p < 0.0001$ and $\text{PhyCol} = 5.19 - 0.04 \text{TSI}_{\text{SD}}$; $R^2 = 0.50$ and $p < 0.0001$) (Figure 5.4). All the sites were included within the prediction limits of the model except for the cases of Lake Lysimachia in September 2016, Lake Paralimni in July 2017 and Lake Volvi in August 1985. Both lakes Lysimachia and Paralimni are shallow with sediment resuspension affecting the TSI_{SD} as indicated by microscopy analysis of lake water; while in the cases of the deep lakes Vegoritis and Amvrakia, they have suffered from drastic water level changes within the last decade, which might also affect the TSI_{SD}.

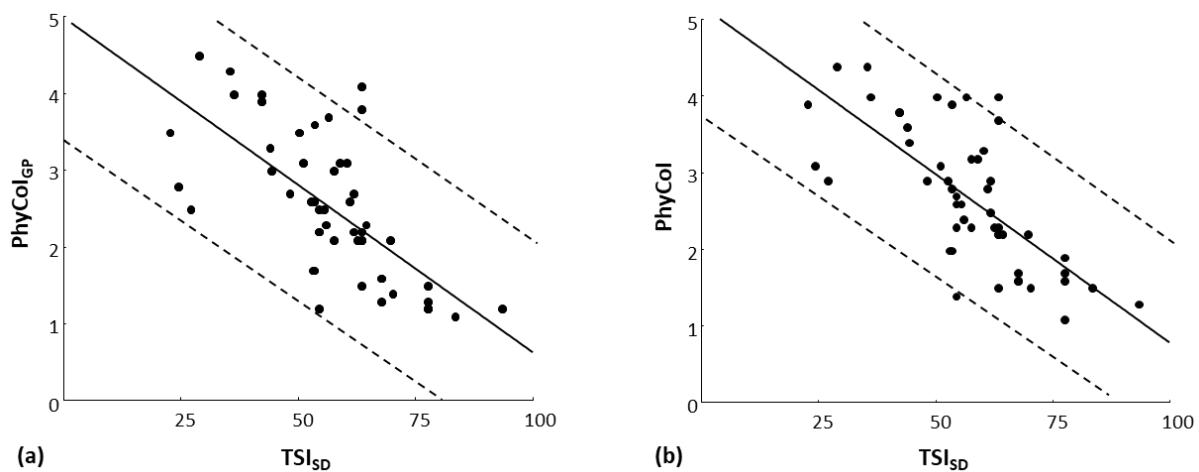


Figure 5.4 Scatter plot of the indices (a) PhyCol_{GP} and (b) PhyCol index against TSI_{SD}. The solid line indicates the linear regression line. The dashed lines indicate the (95%) prediction limits of the model

5.5 Discussion

Grazing Potential across Lake Trophic Spectrum and Ecological Water Quality

This is the first application of the functional index grazing potential (GP) in the Mediterranean region; until now it has been used for temperate lakes (Jeppesen et al. 1997, Arp and Deneke 2007, Arp et al 2010). Moreover, it is a first attempt to evaluate its use for assessing ecological water quality of lakes using at the same time the phytoplankton based PhyCol index. So far, GP has been only correlated with eutrophication, in temperate lakes decreasing across the trophic gradient (Jeppesen et al. 1997), which nevertheless was not the case in the present study. Despite the slightly significant correlation of GP with TSI_{SD}, the range of GP values had overlaps among the categories of trophic state and did not decrease along the trophic gradient.

As for the ecological water quality assessment, the GP significantly correlated with PhyCol index, used for the estimation of ecological water quality and distinguished among the different ecological water quality classes (as estimated using PhyCol classification). However, range overlaps were recorded among the classes. GP depends both on zooplankton and phytoplankton communities, thus it can exhibit high values either when zooplankton is dominated by large individuals (cladocerans or calanoid copepods) or when phytoplankton biomass is low (e.g. in the oligotrophic Lake Amvrakia) or dominated by non-edible species. In the studied lakes, high GP values due to high zooplankton biomass were recorded in cases of good and moderate ecological water quality, such as Lake Kastoria, Lake Vegoritis and Lake Mikri Prespa. For example, in Lake Kastoria (July 2016) high GP values were recorded due to the domination of *D. galeata* (69% contribution to total zooplankton biomass), coinciding with an extreme value of good ecological water quality. Lake Kastoria is an urban eutrophic lake under restoration and the assessment of good quality is restricted to a specific time period reflecting the restored lake plankton community (Katsiapi et al. 2013), explaining the high GP value only for one month during the summer period. Lake Vegoritis (2017) and Lake Mikri Prespa (1992) exhibited high values of GP due to the domination of large-bodied crustacean either of calanoid copepods or their co-dominance with the cladocerans *Diaphanosoma* and *Daphnia*. Domination of daphnids in late spring can lead to the spring clear water phase and when followed by increased biomass of daphnids and calanoids the increased water clarity can be extended during the summer (Lampert et al. 1986, Lathrop et

al. 1999). This extended clear water phase is the outcome of an affective grazing pressure on the entire phytoplankton size spectrum performed by zooplankton, since calanoids and cladocerans are complementary feeding on different size spectra of phytoplankton (Sommer and Sommer 2006). On the other hand, higher GP values where recorded in lakes Voulkaria (September 2016) and Pamvotis (August 2016) (extreme and outlier, respectively) compared to the rest cases of the poor quality class due to high zooplankton biomass, which was nevertheless dominated by the small bodied cladoceran genera *Bosmina* and *Chydorus* coinciding with cyanobacterial blooms. These lakes, as most of the eutrophic Mediterranean freshwater lakes can exhibit prolonged cyanobacterial blooms (up to eight months), with cyanobacterial domination up to more than 90% of the total phytoplankton biomass (Vardaka et al. 2005). In systems with cyanobacterial blooms, small and selective grazing taxa such as *Bosmina*, *Chydorus*, cyclopoid copepods, and rotifers can reach high numbers (Vijverber and Boersma 1997, Hansson et al. 2007).

The low values of GP (<40) and thus the outliers of the low values of good ecological water quality class as well as the lower quality classes (moderate and poor) correspond to either increased phytoplankton biomass or low zooplankton biomass. Based on that, the eutrophic and hypertrophic lakes were expected to have low GP values due to high phytoplankton biomass as recorded in temperate lakes (Jeppesen et al. 1997). This was also recorded in the studied hypertrophic lakes, which are also characterized by poor ecological water quality according to PhyCol. Especially, Lake Voulkaria in July (2016), had the highest GP value recorded while exhibiting the highest phytoplankton biomass recorded in the present study. As for the cases with low zooplankton biomass dominated by small-bodied species they could indicate the impact of planktivorous fish predation (Brooks and Dodson 1965, Jeppesen et al. 2000). Planktivorous fish and their pressure on zooplankton community have been described for Greek lakes; typical examples are the case of *Alburnus volviticus* and *Alosa macedonica* in Lake Volvi (Kleanthidis and Sinis 2001, Bobori et al. 2013) and *Atherina boyeri* in Lake Trichonis (Chrisafi et al. 2007), which could explain the low values of GP in these two lakes. Moreover, strong trophic cascading effects of fish, even without planktivorous species, resulting from dominance of omnivorous and benthivorous fish species with frequent spawning and absence of efficient piscivores have been related with the lack of large-bodied zooplankton grazers in the Mediterranean region (Beklioglu et al. 2007). Extended reproductive period of fish with high fecundity leads to the continuous numbers of fish larva

and juveniles enhanced by species with ontogenetic shifts in fish diet (Mehner and Thiel 1999, Meerhoff et al. 2007). Additionally, the predation of omnivorous species can be important especially in more eutrophic lakes (Drenner et al. 1996) and lower latitudes (Jeppesen et al. 2010) showing feeding plasticity by adapting their diet due to prey availability in each season (Bobori et al. 2013). This type of increased predation could be responsible for the low GP values in the cases of lakes Megali and Mikri Prespa having high production of the omnivorous species *Pseudorasbora parva* and *Lepomis gibossus* (Petriki 2015, Varveris et al. 2016). The impact of *P. parva* on zooplankton has already been described for Mikri Prespa (Rosecchi et al. 1993) while fish predation must have increased after the introduction of *L. gibossus*, a species known for its zooplankton preference depending both on body size and food availability (Rezsú and Specziar 2006, Almeida et al. 2009). Thus, the introduction of *L. gibossus* may explain the lower GP values recorded in Lake Mikri Prespa in 2016 compared to the years 1991-1992.

Considering the above, it is important to identify how the GP values are derived and how differences in zooplankton community composition explain the outliers and extreme values of the ecological water quality classes.

PhyCol_{GP} Application Versus PhyCol

The use of functional traits in environmental assessments has proven a promising and effective approach for studying the link between plankton communities' structures and ecosystem functioning (Weithoff 2003, Baird et al. 2008, Hébert et al. 2017). However, functional metrics are rarely included in the indices used by the Member States for the ecological water quality assessment according to the WFD (Poikane et al. 2015). GP can be used as a plankton community metric, combining the functional trait characteristics of both zooplankton and phytoplankton communities and for this it was used in the present study for modifying PhyCol, a phytoplankton-based index developed for ecological water quality assessment.

The inclusion of GP in the PhyCol index and the restructuring of the latter so as to track besides eutrophication impacts, changes in the fish community resulted in the introduction of a modified plankton index, the PhyCol_{GP} index. PhyCol_{GP} may prove an effective and promising tool as an initial plankton food web index for the ecological water quality assessment of lakes as indicated by its application to the 13 Greek lakes of different trophic

status. It successfully assessed the ecological quality of the studied lakes, since, it not only assessed the same ecological water quality as the PhyCol index but also identified cases with particularities. Such case is the improvement in the ecological water quality of Lake Kastoria in June and July 2016 after the implementation of the lake water flushing method (Moustaka-Gouni et al. 2018). Another case was the moderate quality of Lake Paralimni in September 2017 (classified as good by the PhyCol Index) being in agreement with the increased cyanobacterial biovolume and dominance of *Microcystis aeruginosa* and *Microcystis panniformis* (Moustaka-Gouni et al. 2019b). Similarly, the lower quality indicated by PhyCol_{GP} for Lake Trichonis in September of 2016, coincides with cyanobacterial dominance in phytoplankton biovolume up to 98%. Increased cyanobacteria biovolume is well-known for adverse effects on water quality of freshwater systems, such as impairment to recreational uses, reduced aesthetics, lower dissolved oxygen concentrations, the production of toxins, which can impact aquatic and terrestrial wildlife and human health (Brooks et al. 2016).

Special mention should be made for the case of Lake Megali Prespa, a Greek transboundary lake shared by Greece, North Macedonia and Albania. PhyCol indicated good quality when the mean summer value was used. and the proposed class boundaries for TB metrics for Mediterranean lakes However, even though Lake Megali Prespa is located at the Mediterranean ecoregion, climatologically it belongs to the Alpine biogeographical region (EEA 2019). Based on this differentiation the TB boundaries according were adapted (as in Stamou et al. 2019b) compared to the one based on (Katsiapi et al. 2016b). Katsiapi et al. (2020) applied PhyCol_{GP} in yearly data from Lake Megali Prespa and its ranged from 2.6 to 4.3 (2015-2016) classifying Lake Megali Prespa in moderate water quality (average index value 2.9). Generally, using the boundaries proposed for the Alpine lakes, the ecological water quality for Lake Megali Prespa is clearly lower than good (Moustaka-Gouni et al. 2019b), while signs of its degradation have been reported in the last decade (Katsiapi et al. 2012). Evidence of the moderate quality of Lake Megali Prespa in July 2016 was a conspicuous cyanobacterial *Dolichospermum (Anabaena) lemmermannii* bloom known for its ability to produce various toxins. It is therefore important to mention that PhyCol_{GP} was developed based on data of Greek lakes, thus its boundaries should be adapted when applied in different climatic zones in order for it to be efficient. Such is the case of Lake Megali Prespa where PhyCol_{GP} successfully assessed the ecological water quality after adapting TB boundaries for the alpine zone (Katsiapi et al. 2020).

According to Hering et al. (2006) an effective multi-metric index and its metric should be correlated with eutrophication to enhance its reliability and robustness. Acknowledging that TSI_{SD} index is commonly used as a low-cost and easy measure of eutrophication despite being influenced both by phytoplankton abundance and non-algal particulate matter (Carlson 1977) was used to test the robustness of PhyCol_{GP}. Even though, GP had a slightly significant correlation with eutrophication as indicated by TSI_{SD}, its combination with PhyCol resulted in PhyCol_{GP} having a strong correlation with TSI_{SD} (except for two lakes with high non-algal turbidity). However, phytoplankton biomass is a more appropriate measure of eutrophication (Lyche-Solheim et al. 2013) coinciding also with the TB metric having the higher score for PhyCol_{GP}. Thus, ecosystem monitoring cannot be based only on Secchi Depth measurements, especially for cases with increased organic material lowering transparency (e.g. reservoirs). Thus, a cost-effective monitoring for natural lakes in the Mediterranean region is proposed to have a high frequency Secchi Depth measurements combined with low frequency of PhyCol_{GP} determinations, especially during the warm period.

Of course, the PhyCol_{GP} index still incorporates the same potentials and limitations described for PhyCol (Katsiapi et al. 2016b) such as the combination of several metrics covering the composition and function of plankton communities reflecting complementarily the environmental changes. Even though it might seem that PhyCol_{GP} is a more complex and time-consuming index, further including data of zooplankton communities, it is worthy due to its sensitiveness in cases such as the eutrophic Lake Kastoria under restoration. This indicates the potential use of PhyCol_{GP} not only for monitoring purposes but also during the implementation of restoration measures to prevent further deterioration and protect lakes. The PhyCol_{GP} is in agreement with the ecological foundation of WFD, using metrics related to phytoplankton biovolume, taxonomic composition (Nygaard species), sensitive/insensitive taxa (Quality Group species) even without using EQR methodology, as a useful tool for the associated remedial measures in restoring eutrophic lakes in the River Basin Management Plans. So far, PhyCol_{GP} has only been tested on 13 lakes in Greece. It would be desirable to expand testing to other lakes, both in the Mediterranean climate as well as in other temperate areas. A multimetric index as the PhyCol_{GP}, combining data of the plankton community, can be the base for the establishment of a fully functional plankton index balancing both communities' importance in lake ecosystems regarding metric/sub-indices weights and scores. For such a balanced index, more lake-year data are needed; moreover, the role of

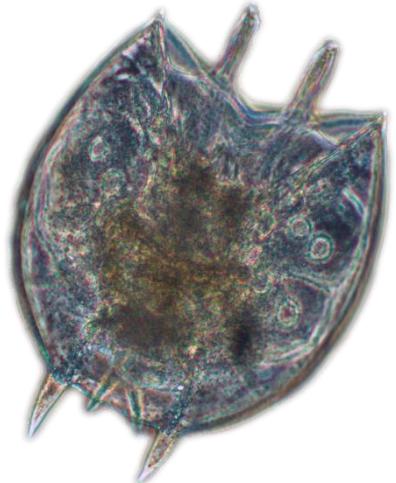
distinctive zooplankton features (e.g. morphological: the role of large bodied cladocerans/crustaceans, functional: the ratio of calanoids to cyclopoids and cladocerans) in explaining water quality deterioration in lakes needs to be further examined.

5.6 Conclusions

In this study, it was shown that the GP index applied on plankton communities of natural lakes during the summer period is efficient in discriminating ecological water quality (high, good, moderate and poor) based on the functional traits of both plankton communities weighting copepods as partially inefficient grazers and some phytoplankton groups of poorly edible algae. Lakes with high GP values have high zooplankton biomass dominated by large cladocerans or/and calanoids while lakes with low GP values are characterized by increased phytoplankton biomass and/or by small bodied zooplankton indicating intensive fish predation. Thus, it is important to identify how the GP values are derived and how differences in zooplankton community composition explain the outliers and extreme values of the ecological water quality classes. Nevertheless, it could potentially be used in a multi-metric index of water quality combining zooplankton and phytoplankton data, since it can be estimated in an easy and cost-effective way considering that the index rely on group level (low identification skills) and both phytoplankton and zooplankton communities are easy and inexpensive to sample and can be sampled in the same time.

Based on the above, GP was included in a modified version of PhyCol, the PhyCol_{GP} index. The PhyCol_{GP} successfully assessed the water quality of the studied lakes evaluated by PhyCol appearing more sensitive in some cases. At this end, PhyCol_{GP} through the combination of different plankton metrics covering various aspects of the structure and function of the plankton community aims to capture the complexities of the lake ecosystem yet remain simple enough to be easily and routinely used. Thus, PhyCol_{GP} can be used in ecological water quality assessment and restoration monitoring programs where it can be also combined with high frequency monitoring of water clarity.

6. Zooplanktonic-Index of Water Quality



6.1 Abstract

6.2 Introduction

6.3 Material & Methods

6.4 Results

6.5 Discussion

6.6 Conclusions

6.1 Abstract

Zooplankton omission from WFD led to the reduction of research on zooplankton-based ecological water quality indices for European lakes. The aim of the present study was to develop an easy-to-use and effective zooplanktonic index for the assessment of the ecological water quality of Mediterranean lakes. As a first step nine quantitative metrics of abundance, biomass, morphometric (mean size of groups) and functional traits (crustacean ratio and large-cladoceran ratio) were applied on data from 13 natural Greek lakes. The four final metrics, A_{Zoo} , B_{Zoo} , MW_{Zoo} and R_{Clad} were selected as those correlated with eutrophication or/and ecological water quality, being numerically appropriate and not having strong inter-correlation. A three level-scoring system and its thresholds were established based on the “data driven methodology” and the expert judgement based on literature. The ZOOplanktonic-Index of water Quality (Zoo-IQ) was calculated as the sum of the score of the final metrics equally weighted and boundaries were proposed in order to be used in a five-grade classification scheme. The application of the Zoo-IQ to the Greek lakes showed that Zoo-IQ index successfully assessed the water quality of the studied lakes characterising almost all hypertrophic lakes with poor and deep lakes with good ecological water quality and identified cases with particularities. Thus, Zoo-IQ is proposed to be used in ecological water quality assessment, restoration and monitoring programs of natural Mediterranean lakes.

Περίληψη

Η παράλειψη του ζωοπλαγκτού από την Οδηγία 2000/60 οδήγησε στη μείωση της έρευνας για δείκτες οικολογικής ποιότητας των υδάτων με βάση το ζωοπλαγκτό για τις ευρωπαϊκές λίμνες. Σκοπός της παρούσας έρευνας ήταν η ανάπτυξη ενός εύχρηστου και αποτελεσματικού ζωοπλαγκτικού δείκτη για την εκτίμηση της οικολογικής ποιότητας των υδάτων των Μεσογειακών λιμνών. Ως πρώτο βήμα εφαρμόστηκαν εννέα ποσοτικές μετρικές της αφθονίας, βιομάζας, μορφομετρικών (μέσου μεγέθους ομάδων) και λειτουργικών χαρακτηριστικών (ο λόγος των καρκινοειδών και ο λόγος μεγαλόσωμων κλαδοκερωτών) σε δεδομένα από 13 φυσικές ελληνικές λίμνες. Από αυτές επιλέχθηκαν οι τέσσερις τελικές μετρικές A_{Zoo} , B_{Zoo} , MW_{Zoo} και R_{Clad} , οι οποίες συσχετίστηκαν με τον ευτροφισμό ή/και την οικολογική ποιότητα του νερού, ήταν αριθμητικά κατάλληλες και δεν είχαν ισχυρή

αλληλοσυσχέτιση. Ένα σύστημα τριών επιπέδων βαθμολόγησης και τα κατώτατα όριά του καθορίστηκαν με βάση τη "μεθοδολογία που βασίζεται στα δεδομένα" και την κρίση εμπειρογνωμόνων με βάση τη βιβλιογραφία. Ο Ζωοπλαγκτικός Δείκτης ποιότητας Υδάτων (Zoo-IQ) υπολογίστηκε ως το άθροισμα των βαθμολογιών των τελικών μετρικών με ίση βαρύτητα και προτάθηκαν όρια για να χρησιμοποιηθούν σε ένα σύστημα ταξινόμησης πέντε επιπέδων. Η εφαρμογή του Zoo-IQ στις ελληνικές λίμνες έδειξε ότι ο δείκτης Zoo-IQ αξιολόγησε με επιτυχία την ποιότητα των υδάτων των εξεταζόμενων λιμνών, χαρακτίρισε σχεδόν όλες τις υπερεύτροφες λίμνες ως ελλιπείς και τις βαθιές λίμνες με καλή οικολογική ποιότητα των υδάτων και αναγνώρισε περιπτώσεις με ιδιαιτερότητες. Έτσι, ο Zoo-IQ προτείνεται να χρησιμοποιείτε σε προγράμματα αξιολόγησης της οικολογικής ποιότητας των υδάτων, αποκατάστασης και παρακολούθησης των φυσικών λιμνών της Μεσογείου.

6.2 Introduction

Biological monitoring provides a direct picture of a lake's status and is much easier understood by the public than chemical data alone (Lyche-Solheim et al. 2013). Different legislation acts for the assessment of the ecological integrity of surface waters (such as WFD and Clean Water Act) resulted in an intense effort for the development of various biotic indices based on different BQEs. Biotic indices provide information on the interaction between the biota and water for the ecological assessment of the surface waters. Among potential biological assessment tools, multi-metric indices are commonly used by combining different categories of metrics/sub-indices (e.g. taxonomic richness, abundance, biological and functional traits), that effectively respond to different aspects of environmental conditions. The combination of relevant metrics is more efficient providing integrated assessments of water quality (Hering et al. 2006). The first multi-metric approach was developed by Karr (1981) for fish communities, while various indices have been developed following Karr's rationale on the basis of different biological communities [e.g. for fish (Harris and Silveira 1999, Harrison and Whitfield 2004), for benthic macroinvertebrates (Barbour et al. 1996, Ferreira et al. 2006), for phytoplankton (Maulood et al. 2011), for zooplankton (Carpenter et al. 2006)].

In Europe, WFD did not include zooplankton as a BQE and consequently research on zooplankton metrics and multi-metric indices is restricted. The only zooplankton index

developed in Europe is ZRTI (Montagud et al. 2019) is not a multi-metric index but it is calculated based on optimal or tolerant values (weighed values) for the dominant species of the studied region according to their correlation with environmental data. However, zooplankton metrics based on abundance, biomass, morphometric and functional characteristics have been used efficiently in long-term monitoring programs of lakes (e.g. Haberman and Haldna 2014). Abundance (A_{Zoo}) and biomass (B_{Zoo}) metrics reflect the theory of trophic cascade; increased nutrients result in a bottom-up control first on phytoplankton while providing greater potential for zooplankton production in response to greater food availability (Carpenter et al. 1985, McQueen et al. 1986). Morphometric metrics based on the mean size of zooplankton groups (MW_{Rot} , MW_{Clad} , MW_{Cop} and MW_{Cru}) or the zooplankton community (MW_{Zoo}) are indicative of pressures due to both eutrophication (Karabin 1985) and fish predation (Brooks and Dodson 1965). Functional metrics based on feeding mode such as R_{Clad} (the ratio of large cladocerans to total cladocerans abundance) tracks shifts in biomass domination of different functional groups such as daphnids, bosminids and chydorids (Barnett et al. 2007) reflecting changes in phytoplankton communities and can provide critical information for the functioning of the food web. R_{Zoo} (ratio of calanoids to cladocerans and cyclopoids abundance) is also a functional metric, based though on habitat preference, since calanoid copepods are more abundant in oligotrophic systems while cladocerans and cyclopoid copepods dominate the eutrophic systems (e.g. Patalas 1972, Gannon and Stemberger 1978, Karabin 1985) and it is used as a measure eutrophication processes. Moreover, the above metrics can track both eutrophication and fish predation, thus they can also effectively track short term changes such as sudden fish kills or lake restoration attempts through biomanipulation (Gannon and Stemberger 1978, Jeppesen et al. 2011). Based on the above, it is strongly believed by the international scientific community that zooplankton communities should be used in quality assessment (e.g. Caroni and Irvine 2010, Jeppesen et al. 2011, Haberman and Haldna 2014).

Thus, the aim of the present study was to develop a multi-metric zooplankton index for the assessment of the ecological water quality of natural lakes in the Mediterranean region. In order to do that it was first necessary to select the final metrics from a group of candidate metrics based on the variability of abundance, biomass and the body size of the main zooplankton groups and functional ratios that were correlated with eutrophication or ecological water quality assessment. For this, nine zooplankton metrics were applied on data

6. Zooplanktonic-Index of Water Quality

from zooplankton communities of 13 natural Greek lakes covering the whole trophic spectrum and the metrics were correlated with eutrophication and ecological water quality. Furthermore, in order to propose a new index, namely Zooplanktonic-Index of water Quality (Zoo-IQ), a scoring system for each of the final metrics had to be developed, so as the final index to be the sum of the score of the final metrics. Finally, the range of the Zoo-iQ index was divided in five parts in order to provide a five-grade ecological classification scheme.

6.3 Material & Methods

In order to find the appropriate metrics for the development of a multi-metric index based on zooplankton communities nine commonly used metrics in monitoring assessments were applied on zooplankton community data during the summer period (from June to September) from 13 natural Greek Lakes. The data of a) lakes Kremasta and Tavropos were not included due to being reservoirs, b) Lake Ozeros were not used due to being dominated by mollusc larvae and c) Lake Megali Prespa due to being the only alpine lake and the current study aim to develop an index for ecological water assessment of natural Mediterranean lakes.

The development of the index consisted of three parts, first the selection of the final (core) metrics among nine candidate metrics, the calibration of the final metrics into a three-grade scoring system and the establishment of their thresholds and finally their combination into the Zooplanktonic-Index of water Quality (Zoo-IQ) and its boundaries for a five-grade (i.e. high, good, moderate, poor, bad) classification scheme.

Metric selection

Zooplankton metrics have been applied based on the abundance and biomass of the three main groups of zooplankton community (Rotifera, Cladocera and Copepoda). The following metrics have been proposed as promising indicators of eutrophication and ecological water quality assessment (e.g. Moss et al. 2003, Jeppesen et al. 2011, Haberman and Haldna 2014).

- **A_{Zoo}:** Total abundance of zooplankton communities (rotifers, cladocerans and copepods).
- **B_{Zoo}:** Total dry biomass of zooplankton communities (rotifers, cladocerans and copepods).

- **R_{Zoo}**: Zooplankton ratio is the ratio of the abundance of the main crustacean groups of zooplankton communities and it was calculated by the equation (1)

$$R_{Zoo} = \frac{Cal}{Clad+Cy} \quad (1)$$

where: Cal is calanoid copepods, Clad is cladocerans and Cy is cyclopoid copepods; for copepods only the abundances of copepodite stages and adults were used. This ratio was not calculated for lakes (Kastoria, Paralimni, Petron, Volvi, Voulkaria and Yliki) without calanoid copepods contributing to zooplankton abundance.

- **MW**: Mean size (weight) of zooplankton groups is the ratio of dry biomass to abundance and can be estimated for the whole community (**MW_{Zoo}**) or for each group (**MW_{Rot}**, **MW_{Clad}** and **MW_{Cop}** for rotifers, cladocerans and copepods, respectively) and **MW_{Cru}** [for crustaceans (cladocerans and copepods)].
- **R_{Clad}**: Ratio of large sized cladocerans (>500 µm) to total cladocerans abundance.

These metrics were evaluated in order to see if they could be used as candidate metrics for the development of a zooplanktonic ecological water quality index for the Mediterranean region. A candidate metric for the development of a multi-metric index, in general, must show a significant correlation to the stressor gradient, either across the whole stressor gradient or measured for a part of it (e.g. only moderate to high quality sites) (Hering et al. 2006). For this, Linear Regression was applied to test the dependence of the nine zooplankton metrics on a) eutrophication assessed by total phytoplankton biomass (Appendix 4) [the sampling of Lake Voulkaria in July 2016 was not included only in this analysis due to increased phytoplankton biomass (74.88 mg/L) creating an outlier] and b) ecological water quality as assessed by PhyCol (Figure 5.3). Moreover, in order to detect whether the nine zooplankton metrics were differentiated among the classes of a) trophic state and b) ecological water quality, ANOVA and Bonferroni procedure were applied. Weight cases for each parameter were used to reduce bias due to the different number of lakes or samplings in each group.

Based on the above analysis (see results), the metrics (**A_{Zoo}**, **B_{Zoo}**, **R_{Zoo}**, **MW_{Zoo}**, **MW_{Cru}**, **MW_{Cop}** and **R_{Clad}**) that significantly correlated with either eutrophication or ecological water quality were further examined in the process of selecting final metrics for the zooplanktonic index. In the selection of the score metrics two procedures are needed. First, to review the value ranges of the candidate metrics in order to exclude numerically unsuitable metrics, for

example, measures with many outliers and extreme values or many zero values (USEPA 2000, Hering et al. 2006). In this step R_{Zoo} was excluded due to having increased zero values (see results). Second, the candidate metrics which show strong inter-correlations (e.g. Spearman's rho > 0.8) with one another are defined as redundant. One of each pair of metrics having strong inter-correlation should be excluded. Thus, Spearman Rank Correlation was applied to the metrics (A_{Zoo} , B_{Zoo} , MW_{Zoo} , MW_{Cru} , MW_{Cop} and R_{Clad}) in order to exclude metrics with strong inter-correlation (rho > 0.8).

All analyses were performed using IBM SPSS Statistics 25.

Metric scoring system

After the selection of the final metrics (A_{Zoo} , B_{Zoo} , MW_{Zoo} and R_{Clad}), a three-grade scoring system was developed. The scoring of the metrics allowed them to be transformed into standard, comparable scores for their integration in the final index (Franco et al. 2009). This scoring system proposed by Karr (1981) has three scores (1, 3 and 5) based on the distribution of the values within the dataset for each metric. Metrics were rated as good (score 5) when similar to the reference value, fair (score 3) when different from the reference value, or poor (score 1) when substantially different from the reference value (Karr 1981).

In order to use this scoring system, reference values have to be obtained, since none of the studied lakes were undisturbed to be used as "reference sites". Thus, we based the metric calibration on the data driven methodology" developed for datasets without reference sites (Harris and Silveira 1999, Harrison and Whitfield 2004, Franco et al. 2009). According to this method, the data are not specifically selected as being of high quality or derived from the best sites, but it assumes that some sites that are minimally disturbed are included (Harris and Silveira 1999). The "best" values observed in the calibration dataset were used to establish the expectations for each metric. Reference conditions represent the "best" values observed and thus the reference value is established as the mean of the data that fell within the upper quartile when ranking each metric (Figure 6.1) (Harris and Silveira 1999, Harrison and Whitfield 2004, Franco et al. 2009).

After the calculation of the reference values the three-grade scoring system proposed by Karr (1981) should be developed based on a combination of the spread of the data and expert judgment to assess the degree of deviation from reference conditions (European Commission 2003, Harrison and Whitfield 2004, Hering et al. 2006). According to the spread

of the data and the calculated reference values, a score of 5 was assigned to values higher than 90% of the reference value. A score of 3 was given to values between 50% and 90% of the reference value, and a score of 1 was assigned to values <50% of the reference value (Figure 6.1) (Harrison and Whitfield 2004, Franco et al. 2009).

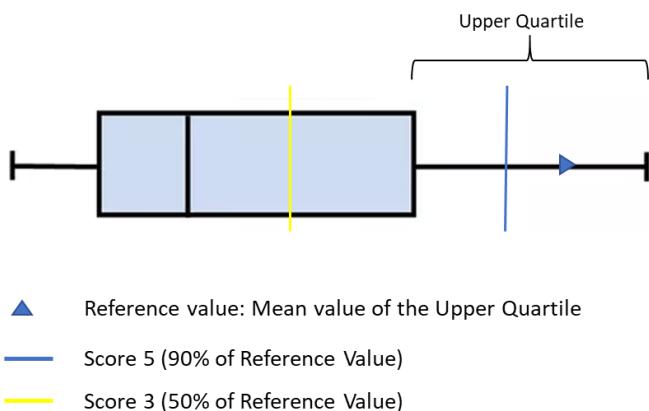


Figure 6.1 Example of the methodology describe for the metric scoring system

This methodology was followed for all four metrics for the establishment of the threshold for score 5. Thus, when the index decreased across disturbance (i.e. MW_{Zoo} and R_{Clad}), a score of 5 was assigned to values higher than 90% of the reference value, while when the index increased across disturbance (i.e. A_{Zoo} and B_{Zoo}) a score of 5 was assigned to values higher than 110% of the reference value. As for the threshold for score 3, it was assigned to values > 50% of the reference value only for the metric MW_{Zoo}. For the metrics A_{Zoo}, B_{Zoo} the lower threshold was very low overestimating the water quality, thus the threshold for score 3 was applied according to literature. For A_{Zoo} the threshold was set at 900 since according to May and O' Hare (2005) oligo-mesotrophic systems have zooplankton abundance up to 900 ind/L due to dominance of rotifers; for B_{Zoo} the threshold was set at 400 since according to Jeppesen et al. (2000) oligotrophic systems have zooplankton biomass (dry) up to 350 µg/L. In the case of R_{Clad} the threshold of score 3 as stricter threshold was set at 0.1 following the lower threshold of Moss et al. (2003) for the Mediterranean lakes.

Index Calculation

Once the three-grade scoring system was developed, each sampling took the respective score for each of the final metrics. The Zoo-IQ was calculated by summing the scores of the four metrics. Thus, Zoo-IQ is calculated as: Zoo-IQ = A_{Zoo} + B_{Zoo} + MW_{Zoo} + R_{Clad}

The Zoo-IQ range is from 4 (all the metrics are rated with 1) to 20 (all the metrics are rated with 5), with a central value of 12 (all the metrics are rated 3). In order to divide this range into the five classes, as indicated in the WFD for the classification of the ecological status, the methodology of Franco et al. (2009) was followed. Index values falling around the central value corresponding to 30% of the total range were rated as moderate, those corresponding to 20% of the total range below and above moderate range were rated as poor' and good, respectively and the rest 15% of the total range were rated as bad' and high, respectively (Franco et al. 2009) (Figure 6.2) . In the case of Zoo-IQ index, the closest natural number was used. Finally, the Zoo-IQ was applied on data from 13 natural Greek lakes, and the mean summer values were used in order to assess their ecological water quality.

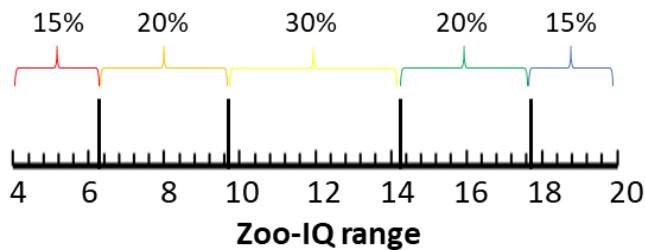


Figure 6.2 Distribution of the boundaries for each ecological state according to Franco et al. (2009) and the color-coding as defined by WFD

Index Evaluation

Zoo-IQ index was applied on zooplankton data (three samplings during the summer period June-September) from lakes Kourna in 2013 (Stamou et al. 2016) and Volvi in 2015 (Katsiapi and Michaloudi 2015) in order to control the application of the index to independent lakes. These lakes were chosen since they were not included in the index development. Their ecological water quality status was assessed based on the mean Zoo-IQ value.

6.4 Results

Metric selection

Nine zooplankton metrics were applied on data from 13 natural Greek lakes and their mean values (\pm standard deviation) are given in Table 6.1.

Table 6.1 Mean values (\pm standard deviation) of the nine metrics from zooplankton communities of 13 Greek lakes. Codes abbreviation according to Table 4.2

Codes	A _{Zoo}	B _{Zoo}	R _{Zoo}	MW _{Zoo}	MW _{Cru}	MW _{Cop}	MW _{Clad}	MW _{Rot}	R _{Clad}
Amv_16	38.64 \pm 15.87	64.49 \pm 22.72	2.29 \pm 2.08	1.70 \pm 0.30	1.81 \pm 0.35	0.93 \pm 0.27	2.08 \pm 0.26	0.12 \pm 0.15	0.43 \pm 0.08
Doi_04	1704.12 \pm 1291.67	274.37 \pm 102.50	0.04 \pm 0.62	0.17 \pm 0.82	0.42 \pm 0.69	0.78 \pm 0.03	0.36 \pm 0.96	0.08 \pm 0.02	0.36 \pm 0.07
Kas_99	1099.49 \pm 354.62	184.87 \pm 121.07		0.16 \pm 0.06	0.44 \pm 0.07	0.83 \pm 0.37	0.40 \pm 0.08	0.06 \pm 0.02	0.37 \pm 0.12
Kas_16	1818.83 \pm 2107.29	202.25 \pm 137.21		0.21 \pm 0.20	0.75 \pm 0.71	1.10 \pm 1.42	0.42 \pm 0.14	0.06 \pm 0.02	0.20 \pm 0.34
Lys_16	1824.94 \pm 1567.65	265.37 \pm 200.60	0.04 \pm 0.02	0.16 \pm 0.03	0.37 \pm 0.05	0.42 \pm 0.04	0.36 \pm 0.06	0.04 \pm 0	0.15 \pm 0.05
MkP_90	471.78 \pm 131.53	324.43 \pm 60.35	0.13 \pm 0.04	0.75 \pm 0.36	0.96 \pm 0.29	0.88 \pm 0.15	1.07 \pm 0.46	0.05 \pm 0.03	0.42 \pm 0.02
MkP_91	478.54 \pm 101.08	206.98 \pm 44.42	0.37 \pm 0.18	0.46 \pm 0.20	0.88 \pm 0.17	0.80 \pm 0.21	0.89 \pm 0.18	0.06 \pm 0.05	0.29 \pm 0.20
MkP_92	255.47 \pm 50.09	214.93 \pm 66.64	0.37 \pm 0.14	0.84 \pm 0.18	0.95 \pm 0.23	0.91 \pm 0.13	0.98 \pm 0.31	0.12 \pm 0.08	0.54 \pm 0.09
MkP_16	85.74 \pm 75.18	29.76 \pm 19.55	1.15 \pm 0.78	0.45 \pm 0.20	0.87 \pm 0.14	0.39 \pm 0.08	0.93 \pm 0.09	0.03 \pm 0	0.06 \pm 0.11
Pam_16	457.76 \pm 476.62	180.78 \pm 165.96	0.08 \pm 0.07	0.43 \pm 0.08	0.75 \pm 0.22	0.77 \pm 0.33	0.77 \pm 0.10	0.07 \pm 0.02	0.37 \pm 0.23
Par_17	1231.94 \pm 381.54	209.98 \pm 41.76		0.19 \pm 0.09	0.36 \pm 0.07	0.42 \pm 0.15	0.35 \pm 0.04	0.13 \pm 0.12	0.12 \pm 0.20
Pet_10	1131.11 \pm 261.46	247.63 \pm 49.83		0.23 \pm 0.07	0.46 \pm 0.04	0.41 \pm 0.09	0.50 \pm 0.10	0.06 \pm 0.01	0.17 \pm 0.16
Tri_16	52.72 \pm 15.21	58.45 \pm 8.58	2.53 \pm 1.25	1.16 \pm 0.33	1.53 \pm 0.39	0.75 \pm 0.11	1.66 \pm 0.32	0.19 \pm 0.08	0.59 \pm 0.16
Veg_87	27.08 \pm 13.20	34.70 \pm 19.20	2.34 \pm 0.70	1.31 \pm 0.33	1.59 \pm 0.44	0.95 \pm 0.11	1.8 \pm 0.53	0.03 \pm 0.02	0.54 \pm 0.12
Veg_17	202.06 \pm 41.66	333.89 \pm 13.28	0.51 \pm 0.42	1.69 \pm 0.28	2.47 \pm 0.47	3.00 \pm 0.40	2.03 \pm 0.65	0.07 \pm 0.07	0.86 \pm 0.04
Vol_84	109.40 \pm 53.33	61.41 \pm 40.44		0.53 \pm 0.11	0.62 \pm 0.07	0.80 \pm 0.22	0.56 \pm 0.11	0.20 \pm 0.06	0.52 \pm 0.08
Vol_85	148.15 \pm 43.30	46.09 \pm 22.84		0.30 \pm 0.08	0.59 \pm 0.13	1.04 \pm 0.34	0.49 \pm 0.03	0.06 \pm 0.02	0.64 \pm 0.07
Vol_86	986.65 \pm 213.30	212.05 \pm 5.56		0.22 \pm 0.05	0.39 \pm 0.06	0.54 \pm 0.08	0.37 \pm 0.5	0.08 \pm 0.02	0.19 \pm 0.05
Vou_16	1104.51 \pm 1458.31	367.90 \pm 492.45		0.30 \pm 0.10	0.49 \pm 0.08	0.37 \pm 0.10	0.93 \pm 0.30	0.05 \pm 0.02	0.10 \pm 0.13
Yli_90	843.17 \pm 271.17	264.33 \pm 102.68		0.31 \pm 0.08	0.59 \pm 0.31	0.59 \pm 0.10	0.63 \pm 0.48	0.05 \pm 0.03	0.10 \pm 0.07

6. Zooplanktonic-Index of Water Quality

These metrics were correlated with eutrophication (Table 6.2) either by having a significant correlation with phytoplankton biomass (Figure 6.3) or by differentiating, even partially, the trophic state categories (Figure 6.4). They were also correlated with ecological water quality (Table 6.3) either by having a significant correlation with PhyCol index (Figure 6.5) or by differentiating, even partially, the ecological water quality classes (Figure 6.6). The metrics A_{Zoo} , R_{Zoo} , MW_{Zoo} , MW_{Cru} and MW_{Cop} were correlated significantly with both eutrophication and ecological water quality and the metrics B_{Zoo} and R_{Clad} were correlated significantly only with eutrophication, while MW_{Clad} and MW_{Rot} were not significantly correlated with either of them. Thus, MW_{Clad} and MW_{Rot} were excluded as candidate metrics (and for the rest of the analysis) for the development of the zooplanktonic index of ecological water quality of Mediterranean lakes.

Table 6.2 Results of the correlation of the nine metric with eutrophication via both linear regression analysis against phytoplankton biovolume and ANOVA analysis against trophic state classes (indicated by mean summer phytoplankton biomass)

Metrics	Phytoplankton biomass (mg/L)			Trophic state	
	Equation	R2	p	F	p
A_{Zoo}	$y = 433.88 + 34.79 x$	0.108	< 0.05	1.292	> 0.05
B_{Zoo}	$y = 133.30 + 7.06 x$	0.138	< 0.01	3.507	< 0.05
R_{Zoo}	$y = 1.22 - 0.06 x$	0.202	< 0.01	3.341	< 0.05
MW_{Zoo}	$y = 0.78 - 0.02 x$	0.139	< 0.01	10.678	< 0.0001
MW_{Cru}	$y = 1.10 - 0.03 x$	0.152	< 0.01	5.887	> 0.01
MW_{Cop}	$y = 1.07 - 0.02 x$	0.105	< 0.05	7.453	< 0.001
MW_{Clad}	$y = 0.96 - 0.01 x$	0.036	> 0.05	2.474	> 0.05
MW_{Rot}	$y = 0.09 - 0.001 x$	0.027	> 0.05	1.614	> 0.05
R_{Clad}	$y = 0.40 - 0.006 x$	0.037	> 0.05	3.191	< 0.05

Table 6.3 Results of the correlation of the nine metric with ecological water quality via both linear regression analysis against PhyCol index and ANOVA analysis against ecological water quality classes (indicated by PhyCol)

Metrics	PhyCol index			Ecological water quality	
	Equation	R ²	p	F	p
A_{Zoo}	$y = 1333.53 - 235.71 x$	0.058	> 0.05	3.349	< 0.05
B_{Zoo}	$y = 303.09 - 41.30 x$	0.056	> 0.05	2.721	> 0.05
R_{Zoo}	$y = -2.13 + 1.02 x$	0.997	< 0.001	7.649	< 0.001
MW_{Zoo}	$y = -013 + 0.27 x$	0.223	< 0.01	32.039	< 0.0001
MW_{Cru}	$y = -0.01 + 0.33 x$	0.245	< 0.01	18.373	< 0.0001
MW_{Cop}	$y = 0.08 + 0.30 x$	0.211	< 0.05	31.201	< 0.0001
MW_{Clad}	$y = 0.40 + 0.16 x$	0.049	> 0.05	0.785	> 0.05
MW_{Rot}	$y = 0.05 + 0.01 x$	0.028	> 0.05	2.798	> 0.05
R_{Clad}	$y = 0.27 + 0.03 x$	0.016	> 0.05	0.984	> 0.05

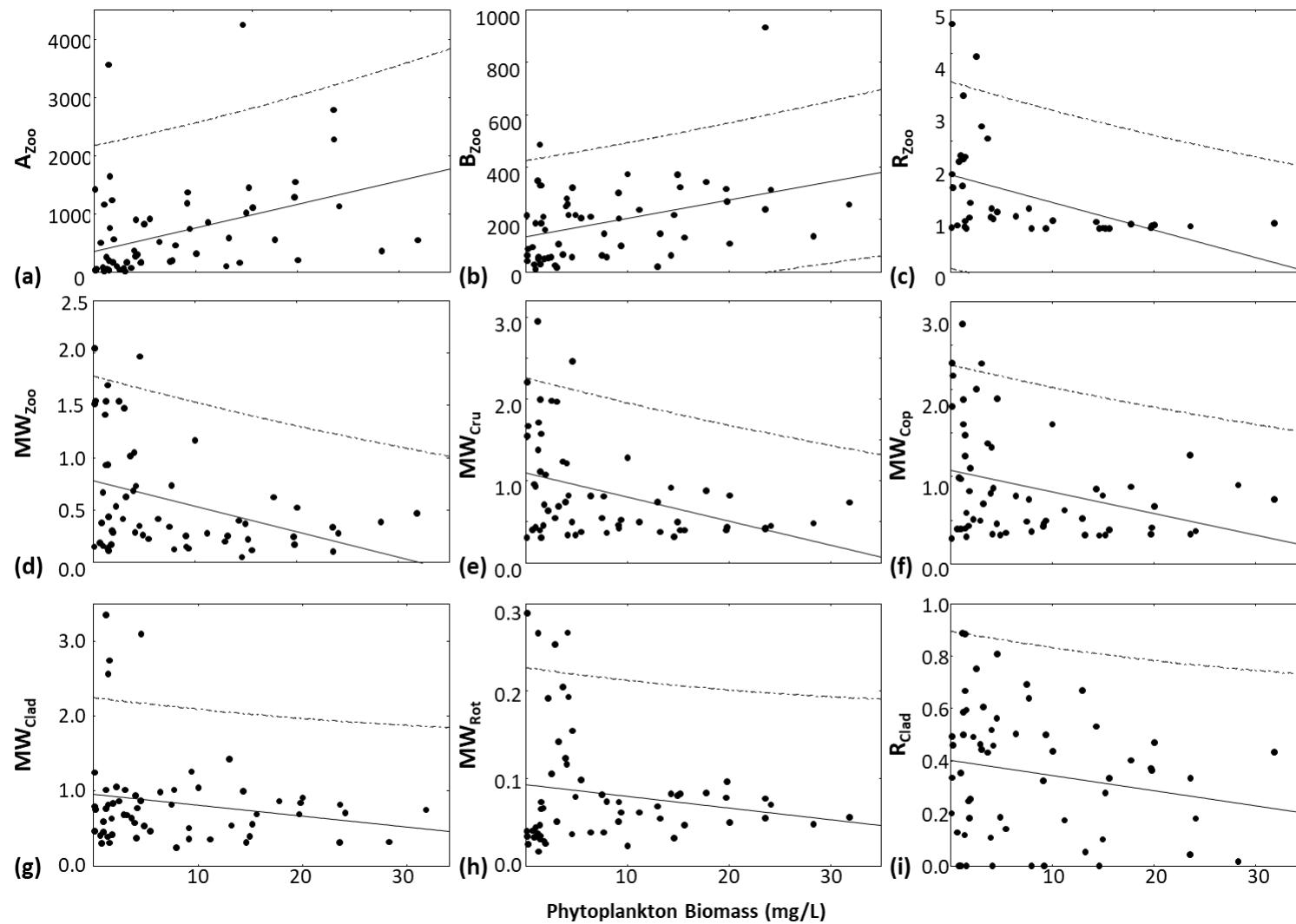


Figure 6.3 Scatter plot of the nine zooplanktonic metrics against phytoplankton biomass. Solid line indicates the linear regression line and dashed lines indicate 95% prediction limits of the model

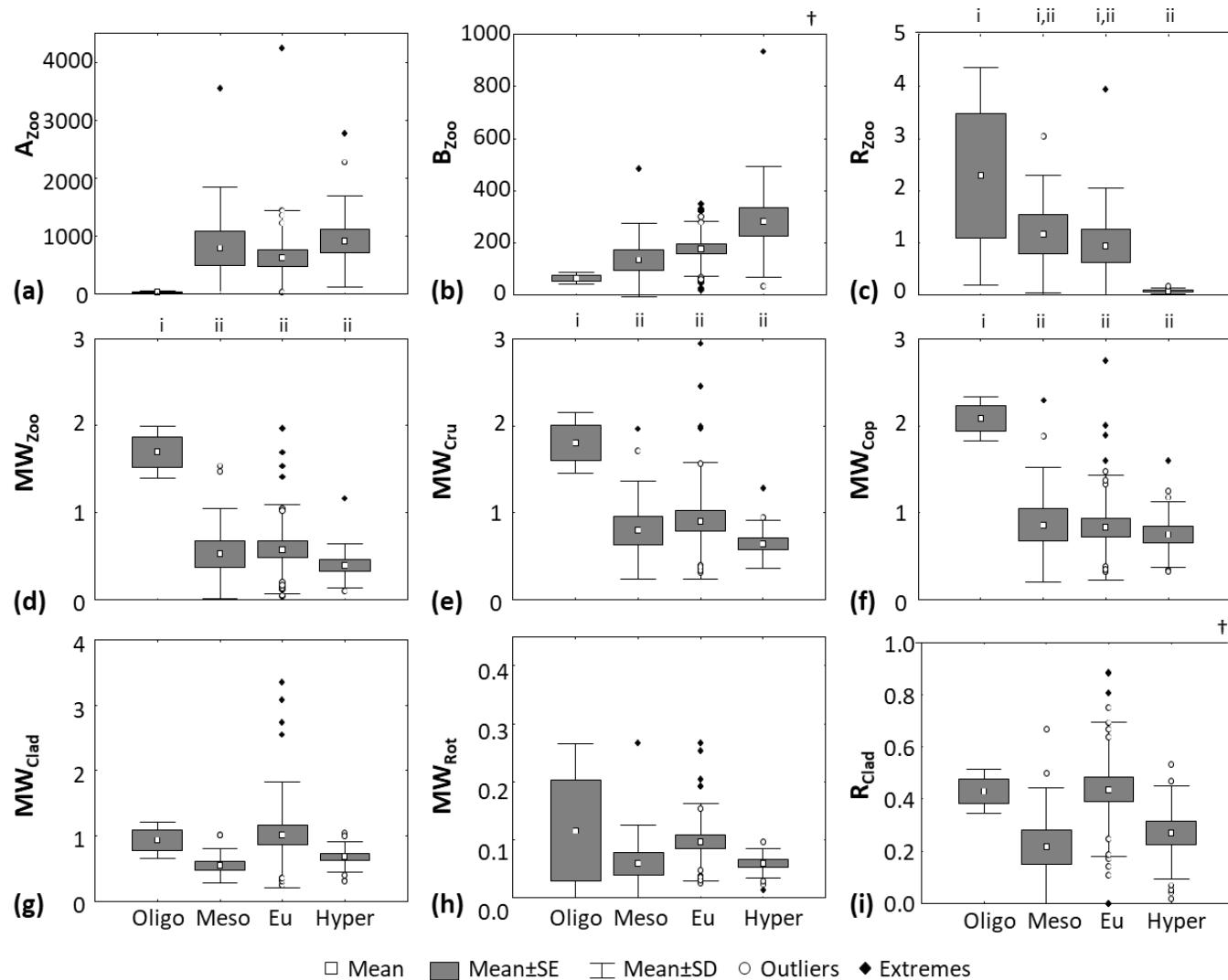


Figure 6.4 Box plots of the nine zooplankton metrics grouped by trophic state (indicated by mean summer phytoplankton biomass). i, ii Significant differences, † none significant difference [Bonferroni procedure applied in cases with $p < 0.05$ (ANOVA)]

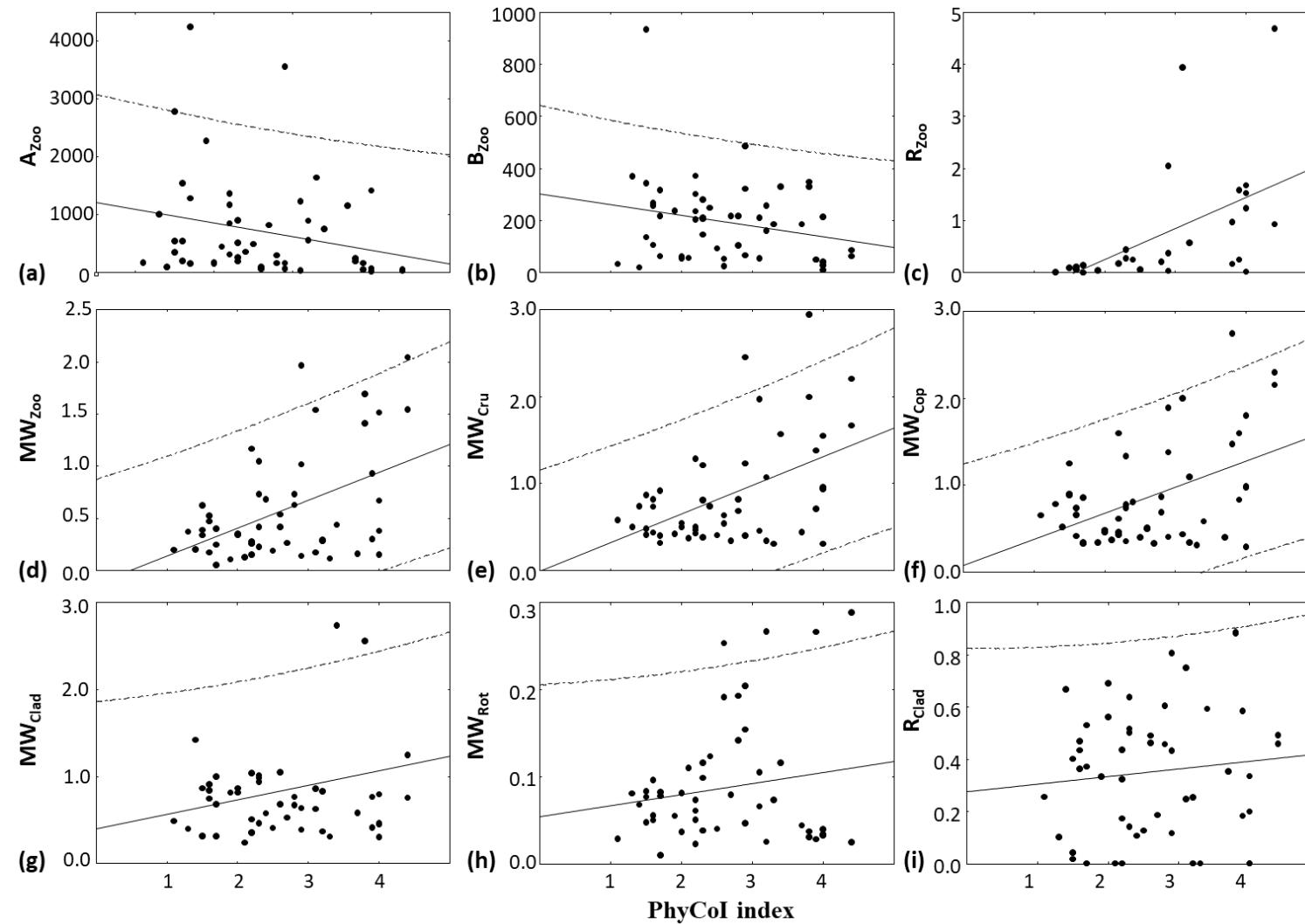


Figure 6.5 Scatter plot of the nine zooplanktonic metrics against PhyCoI index. Solid line indicates the linear regression line and dashed lines indicate 95% prediction limits of the model

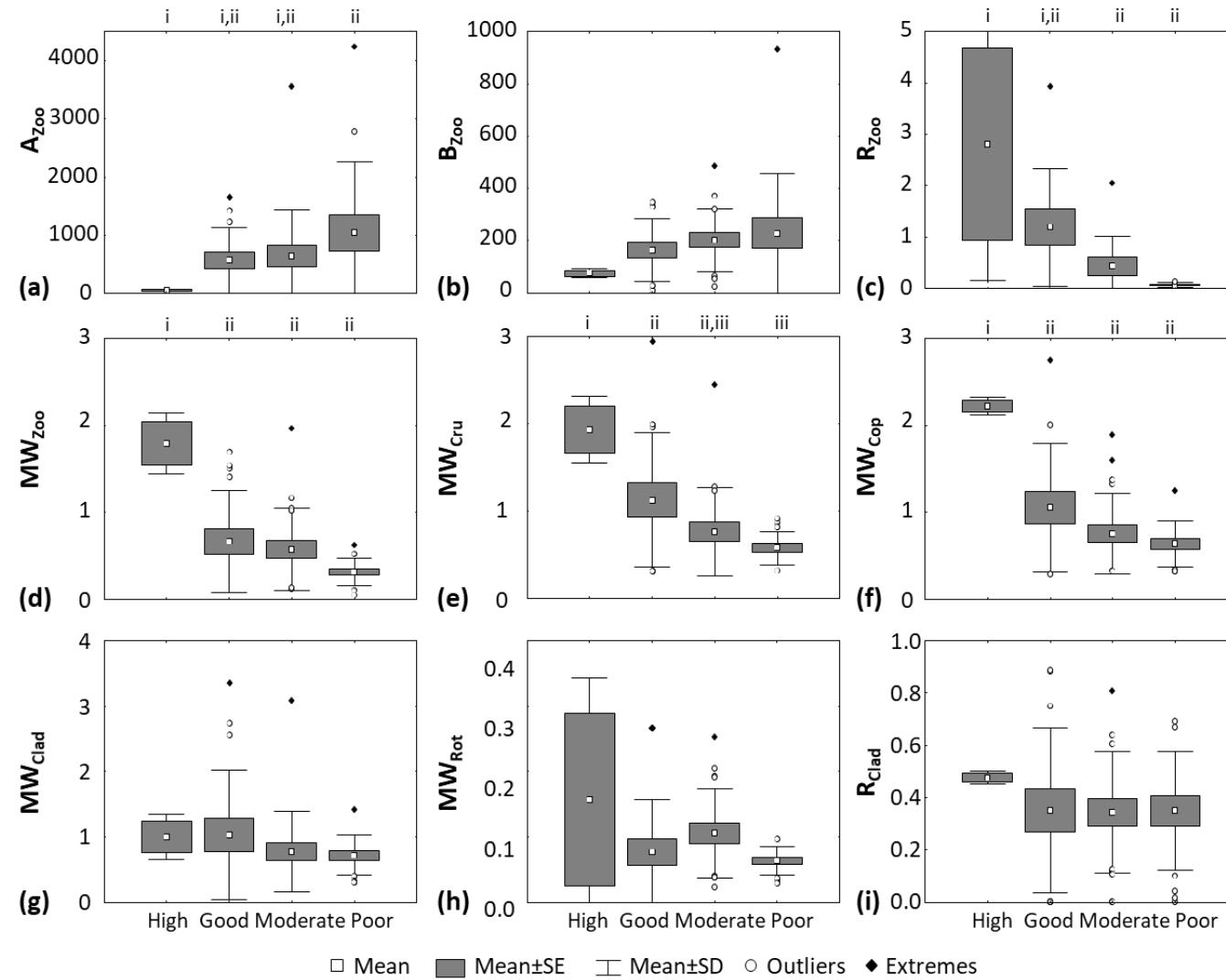


Figure 6.6 Box plots of the nine zooplankton metrics grouped by tr ecological water quality classes (indicated by PhyCol index). i, ii Significant differences [Bonferroni procedure applied in cases with $p < 0.05$ (ANOVA)]

6. Zooplanktonic-Index of Water Quality

The values range of each metric was reviewed according to Table 6.1. The metric R_{Zoo} was then excluded since it was not calculated (indicating zero values) for 45% of the samplings due to lakes without calanoids copepods.

Then Spearman Rank Correlation was applied to the six metrics A_{Zoo} , B_{Zoo} , MW_{Zoo} , MW_{Cru} , MW_{Cop} and R_{Clad} to test their inter-correlation (Table 6.4). Strong inter-correlation was recorded between the metrics MW_{Zoo} and MW_{Cru} ($\rho = 0.874$) and MW_{Zoo} and MW_{Cop} ($\rho = 0.864$). As a result, the metrics MW_{Cru} and MW_{Cop} were also excluded from the candidate metrics. Hence, the A_{Zoo} , B_{Zoo} , MW_{Zoo} , and R_{Clad} are the final metrics for the development of the zooplanktonic index for the assessment of ecological water quality assessment of the natural Greek lakes.

Table 6.4 Spearman Rank Correlation results for the six zooplanktonic metrics. ** Significant correlation at 0.01 level (2-tailed) and bold values indicate high correlation ($\rho > 0.8$)

	A_{Zoo}	B_{Zoo}	MW_{Zoo}	MW_{Cru}	MW_{Cop}	R_{Clad}
A_{Zoo}						
B_{Zoo}	0.704**					
MW_{Zoo}	-0.754**	-0.120				
MW_{Cru}	-0.752**	-0.193	0.874**			
MW_{Cop}	-0.709**	-0.164	0.864**	0.920**		
R_{Clad}	-0.517**	-0.142	0.567**	0.630**	0.453**	

Metric scoring system

In order to develop the scoring system for each of the final metrics that was used according to the “data driven methodology” the calibration of each metric was done using the distribution of the values of each metric as it was established by the Interquartile Range. The reference conditions were established as the mean of the data that fell within the upper quartile when ranking each metric (Figure 6.7). The thresholds for the scores 1, 3 and 5 are also presented in (Figure 6.7).

Zoo-IQ Index Application

The Zoo-IQ was calculated for each sampling of the 13 natural Greek lakes as the sum of the scores of the four final metrics. This application and the boundaries of the index for the classification of the ecological status in five classes are presented in Figure 6.6. Zoo-IQ ranged from 4 (the lower possible value) in the cases of Lake Pamvotis (August 2016) and Lake

Voulkaria (September 2016) to 18 in the cases of Lake Amvrakia (September 2016), Lake Trichonis (September 2016) and Lake Vegoritis (August and September 1987) (Figure 6.7).

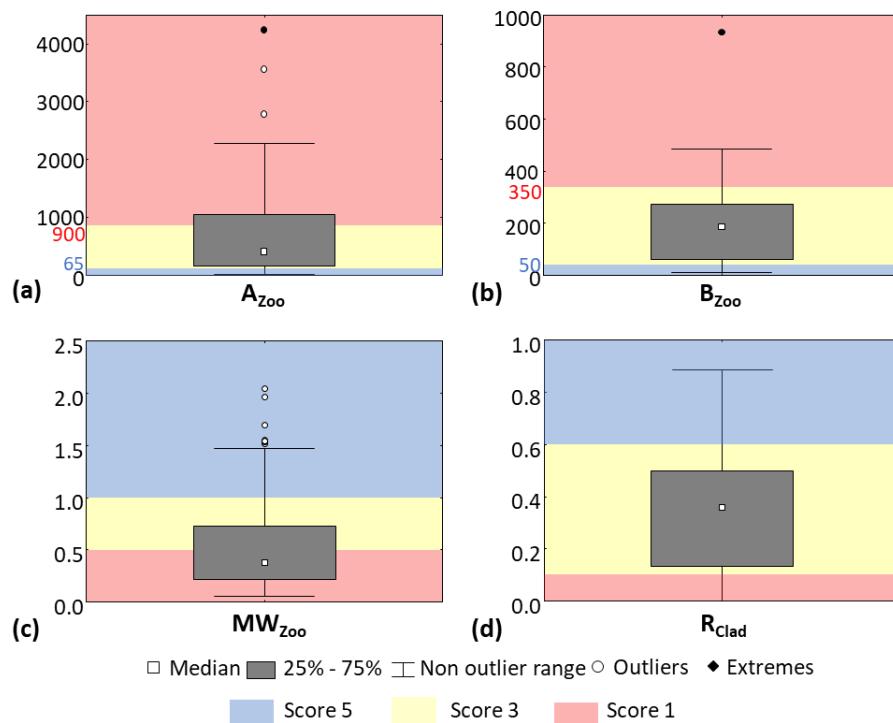


Figure 6.7 Box plot of the final metrics and the ranges for the scores 1, 3 and 5

The ecological water quality of each lake indicated during the same year did not coincide in all cases, for example the ecological water quality of Lake Voulkaria (2016) ranged from moderate to bad while Lake Doirani (2004) was characterized as moderate in all three samplings (Figure 6.8). Finally, the mean summer values were used for the assessment of each lake (Figure 6.9). From the 13 studied lakes, none where characterized with high or bad ecological water quality.

The Zoo-IQ was applied in lakes Kourna in 2013 (10.67 ± 1.15) and lake Volvi (12 ± 0) as well and the ecological water quality status of both lakes was characterized as moderate.

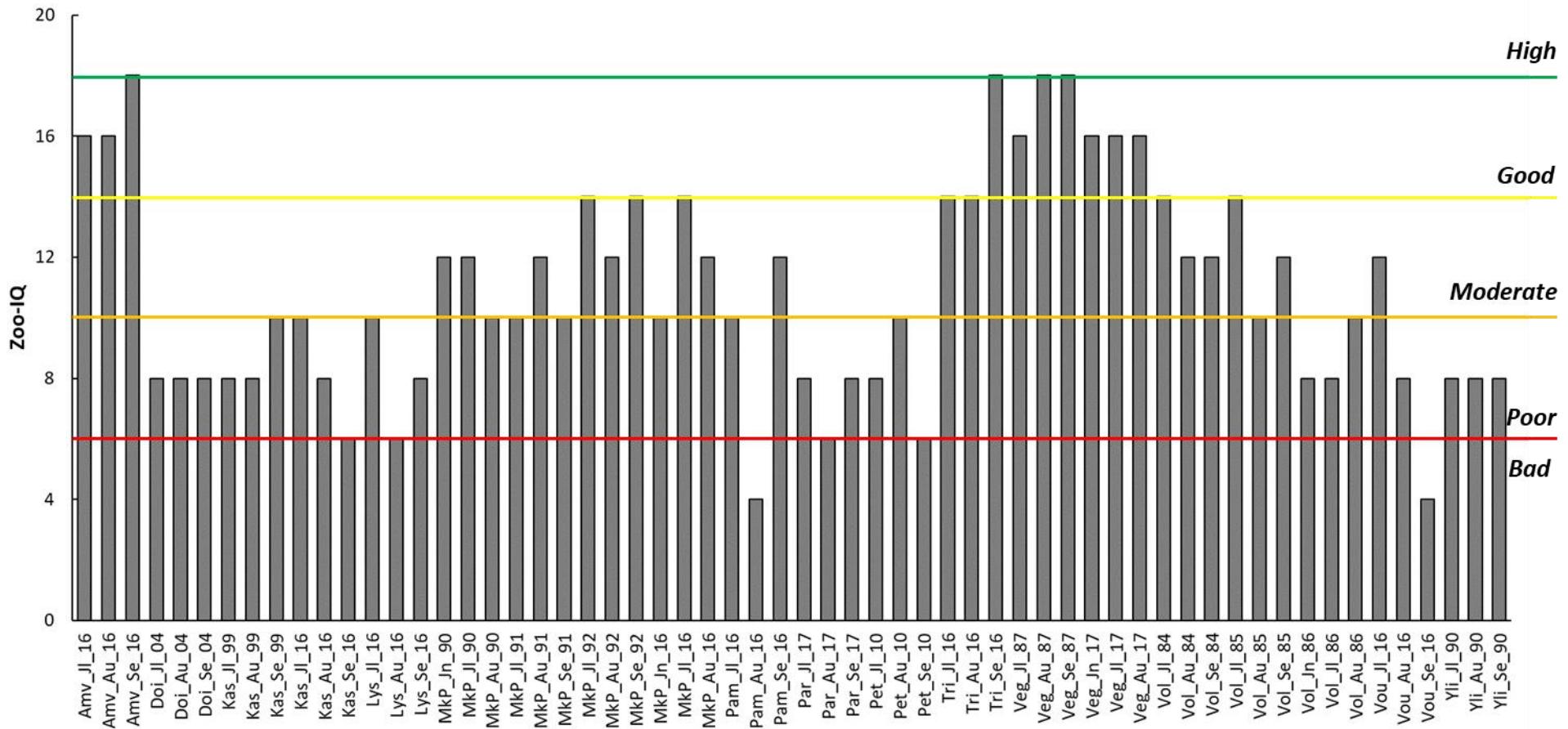


Figure 6.8 Zoo - IQ index values of the 13 Greek lakes in the various samplings. Abbreviations according to Figure 3.1

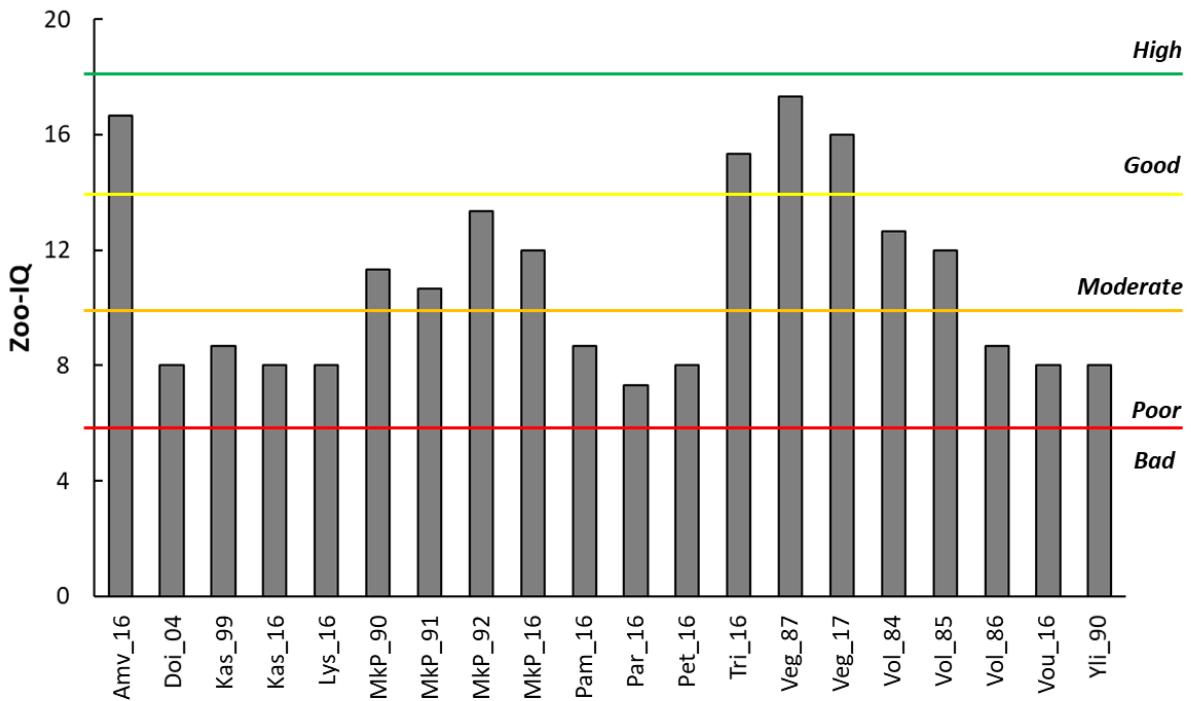


Figure 6.9 Mean summer Zoo - IQ index values of the 13 Greek lakes in the various samplings. Abbreviations according to Table 4.2

6.5 Discussion

Development of the Zoo-IQ

The main goal of the present study was to develop an easy-to-use and effective index for assessing the ecological water quality of lakes based on zooplankton metrics. For this, quantitative and easy to measure zooplankton metrics of abundance, biomass, morphometric and functional traits were evaluated based on data of abundance and/or biomass of the main zooplankton groups for assessing the ecological water quality of lakes based on data from the summer period. Nine commonly used metrics [i.e. total abundance (A_{Zoo}) and biomass (B_{Zoo}), mean size of the different groups (MW_{Zoo} , MW_{Cru} , MW_{Clad} , MW_{Cop} , MW_{Rot}) and abundance ratios of the crustacean groups (R_{Zoo}) and cladocerans size (R_{Clad})] in eutrophication or ecological water quality monitoring in European freshwater systems (e.g. Karabin 1985, Caroni and Irvine 2010, Jeppesen et al. 2011, Ejsmont-Karabin 2012, Ejsmont-Karabin and Karabin 2013, Haberman and Haldna 2014) were evaluated for the development of the Zoo-IQ index. However, only the metrics A_{Zoo} , B_{Zoo} , MW_{Zoo} and R_{Clad} were selected as final metrics for the development of Zoo-IQ index.

The metrics MW_{Clad} and MW_{Rot} were excluded since they did not correlate significantly with either eutrophication or ecological water quality of Greek lakes, while both MW_{Cru} and MW_{Cop} had strong inter-correlation with MW_{Zoo} . The three mean size metrics (MW_{Zoo} , MW_{Cru} and MW_{Cop}) were inter-correlated indicating that they are mainly influenced by copepods; adult copepods are larger than rotifers and small bodied cladocerans dominating in Greek lakes (see chapter 3). The metric R_{Zoo} could not be used in the Zoo-IQ since it was found to be numerically inappropriate (increased zero cases) due to lakes Kastoria, Paralimni, Volvi and Yliki not having calanoid copepods and lakes Petron and Vouklaria where calanoids copepods have been recorded according to Zarfdjian and Economidis (1989) and Stamou (2016), respectively, but were not recorded in the present study. Despite the exclusion of R_{Zoo} from the Zoo-IQ, it is a metric that should be used in monitoring assessment of lakes with calanoids copepods.

The scoring system of the final metrics (A_{Zoo} , B_{Zoo} , MW_{Zoo} and R_{Clad}) was based on the methodology proposed for multi-metric indices by Karr (1981) and for this reference conditions or values should be described. Reference conditions have been criticized for both a) its conceptual approach of ecosystems as stable and predictable and b) the difficulty in recognising reference sites, since they hardly exist today while using present data of reference sites underestimates the continuously evolving systems due to the human impacts on natural systems (Bouleau and Pont 2015, Moss 2008). Specifically, for Greece, the proposed reference sites need to be revised based on typology descriptors such as lake size and other basic features like altitude, salinity, climate factors, and inherent detritus water content (Moustaka-Gouni et al. 2019b). Of major importance are the cases of Lake Kourna and Lake Paralimni which are selected as reference sites for the Greek national monitoring program (European Commission 2018). However, Lakes Kourna and Paralimni, are not appropriate types for determining the status of large deep and shallow Greek/transboundary lakes and do not qualify as representing reference conditions according to Moustaka-Gouni et al (2019b).

Thus, in the present study the data driven methodology" was used for finding the reference values and establishing the thresholds for the three-level scoring scheme (1, 3, 5). Expert judgement was also used for the establishment of the threshold for score 3 of A_{Zoo} , B_{Zoo} and R_{Clad} since these metrics have been mainly used in temperate European lakes. Mediterranean lakes have biological differences from temperate lakes, such as intense fish

predation and extended phytoplankton blooms that both affect zooplankton communities leading to relatively lower zooplankton biomass (Moustaka-Gouni et al. 2014). These differences in plankton communities indicated the need for stricter thresholds for the assessment of Mediterranean lakes. Thus, one of the first schemes developed for the implementation of the WFD, ECOFRAME, proposed stricter thresholds for lakes in warm climates compare to colder climates for R_{Clad} (Moss et al. 2003). This lower threshold for R_{Clad} proposed for Mediterranean lakes (Spain) in Moss et al. (2003) was adopted, in the present study, as the threshold for score 3. Even though there are no proposed thresholds for A_{Zoo} and B_{Zoo} in Europe, it has been found that oligotrophic lakes ranges are below 900 ind/L (May and O'Hare 2005) and 350 µg/L (Jeppesen et al. 2000), respectively. Acknowledging that, oligotrophic lakes' maxima are expected to be even lower in the Mediterranean lakes (Moustaka-Gouni et al. 2014), these values were not proposed as the threshold of score 5 but for score 3.

After the establishment of the thresholds for each metric scoring, the final index was the sum of the four metrics equally weighted. Zooplankton community succession during summer could affect these metrics either by the population increase of rotifers or by changes in the zooplankton community size structure as described in chapter 3. This could indicate that differences in zooplankton community composition and succession even in the same lake could result in a range of the Zoo-IQ value corresponding to different ecological water quality classes, as it was recorded in almost all cases. Thus, the use of the mean summer value of the Zoo-IQ index is proposed to be used in the quality assessment of natural lakes.

Zoo-IQ Index Application

Zoo-IQ Index is the first attempt to develop an assessment tool based on zooplankton communities for natural Greek lakes, and Mediterranean lakes in general. Zoo-IQ could effectively assess ecological water quality trucking besides eutrophication impact, changes in the fish community as well.

Zoo-IQ detected effectively poor ecological quality, since all hypertrophic lakes dominated by small bodied species (Doirani, Pamvotis, Vouklaria and Yliki) except lake Mikri Prespa in 1990 (dominated by larger species) were characterized as poor. In hypertrophic lakes with cyanobacterial blooms, small and selective grazing taxa can reach high abundances (Vijverberg and Boersma 1997, Hansson et al. 2007), such as *Bosmina* and *Chydorus*

(dominated in lakes Pamvotis and Voulkaria), nauplii, and rotifers (dominated in lakes Yliki and Doirani). Even though none of the lakes were characterized as bad (using the mean summer value) there were two cases a) Lake Pamvotis in August and b) Lake Voulkaria in September that were assessed as being of bad water quality. Lake Pamvotis, had cyanobacterial blooms during all summer (Cyanobacterial contribution 98-100%, Appendix 4), scoring the lower Zoo-IQ value in August when *Chydorus sphaericus* dominated (43.5% contribution in abundance), a small bodied cladoceran resulting into scoring 1 for the size metrics (MW_{Zoo} and R_{Clad}). In the case of Lake Voulkaria, a degradation of the ecological water quality was recorded after July (2016) when the highest phytoplankton biomass was recorded (74.88 mg/L), followed by domination of cyanobacteria gradually resulting in high abundance and biomass of both *Bosmina* and *Chydorus* in September.

As for high values of Zoo-IQ, the lakes characterized with good ecological water quality according to Zoo-IQ index were all deep (i.e. Lake Amvrakia, Lake Trichonis and Lake Vegoritis). Deep lakes are favored compared to shallow in terms of water quality due to differentiation in their structure and functioning. For example, nutrients recycling is smaller due to a continuous loss of nutrient to the hypolimnion during summer (Scheffer 1997). Moreover, large deep lakes also favor zooplankton communities due to pelagic and littoral zones and water stratification resulting in niche differentiation (Dodson 1992) favouring the co-domination of various species. Moreover, the large depth enables the diel vertical migration of organisms as an avoidance measure against predation (Lampert 1989). Both lake Trichonis and Vegoritis have planktivorous fish species [*Atherina boyeri* (Chrisafi et al. 2007) and *Alburnus thessalicus* (Petriki 2015), respectively], however their zooplankton communities are dominated by large-bodied species. Especially Lake Vegoritis is the lake inhabited by larger-bodied species due to the calanoid copepod *Neolovenula alluaudi* and the cladocerans *Daphnia* up to 1.5 mm and *Diaphanosoma* up to 1.2 mm (even more than one size class larger than other lakes).

Zoo-IQ also identified cases with particularities due to the implementation of restoration programs, such as lakes Kastoria and Volvi. In the case of Lake Kastoria, the implementation of the lake water flushing method was used as a restoration management plan showing an improvement in the ecological water quality (based on phytoplankton data) of Lake Kastoria in June and July 2016 (Moustaka-Gouni et al. 2018) but not in August. This was also reflected in Zoo-IQ, since after the higher value in July there was a degradation

coinciding with the lower biomass of daphnids. In the case of Lake Volvi the ecological water quality was moderate in 1984 and 1985 but poor in 1986. A translocation of *Perca fluviatilis*, a piscivorous fish, from Lake Doirani was conducted in 1986 (Economidis et al. 2000). After this a trophic cascade was expected (Carpenter et al. 1985), by reducing the numbers of the planktivorous fish *Alosa macedonica*. However, the release from fish predation resulted into the number explosion of nauplii. Thus, the increase of small bodied species in high numbers resulted in lower ecological water quality according to Zoo-IQ index.

Zoo-IQ index was also applied on data not included in the process of its development. Both Lake Volvi in 2015 and Lake Kourna 2013 were characterized as having moderate ecological water quality. This was expected for Lake Volvi since it has been identified as moderate during previous years (1984-1985) (Figure 6.7) and by the use of ecological water quality indices based on different BQES in different studied periods [e.g. by PhyCol_{Gp} based on both zooplankton and phytoplankton data (Chapter 5, Stamou et al. 2019b), PhyCol based on phytoplankton (Katsiapi et al. 2016b), GFLI based on fish (Petriki et al. 2017)]. Lake Kourna was characterized by low abundance and biomass, dominated by rotifers and nauplii in terms of abundance, but also by the absence of cladoceran species in July and August [(only *B. longirostris* was recorded in September (Stamou 2016)]. Cladocerans are the main filter feeders controlling phytoplankton biomass and their absence could not be accompanied with higher ecological water quality status.

Considering the above, it is therefore evident that Zoo-IQ index could be used during ecological water quality assessment of natural lakes since it tracks changes in zooplankton community resulting either from top-down or bottom-up forces. Moreover, it should be used for monitoring purposes during the implementation of restoration plans to prevent further deterioration.

Potential and limitations

Zoo-IQ combines four metrics based on zooplankton community structure and function. The dataset used for the development and testing of the Zoo-IQ index was derived from the extended summer period (June to September) acknowledging the natural variability and succession events of the Mediterranean zooplankton communities, which integrate the uninterrupted growth of summer-autumn phytoplankton alongside the intense fish predation due to the extended reproduction period of fish (Moustaka-Gouni et al. 2014). The

combination of the four metrics in a complementary way in order for Zoo-IQ to incorporate the main aspects of zooplankton community (abundance, biomass, body size as a morphometric trait and large-cladocerans dominance as a functional trait) reflecting zooplankton community responses to environmental changes affecting the food-web functioning. It is generally assumed that combination of several metrics enhances consistency and robustness of an index, as any accidental outliers of one metric can be smoothed by the other metrics (Gabriels et al. 2010).

Zoo-IQ has also the advantage of being an easy to use and effective index. Sampling zooplankton is generally straightforward, less complex and time consuming and inexpensive compared to other BQE's sampling (e.g. fish). At the same time the measurement of the four metrics is easy without needing identification skills and cladoceran size measurements can be easily made under a microscope during abundance calculation. Biomass calculation can be measured either via drying in an oven after the abundance calculation or via length-weight relations in order to be more accurate. In the latter case, identification at least at a genus level is needed. Thus, this avoidance of species identification makes the Zoo-IQ easy and insensitive to expert/personel's identification skills.

Moreover, Zoo-IQ is the only zooplankton index which can be tested without modifications in other lakes since it includes three metrics based on the whole community and one based on cladoceran community. Other already developed zooplankton indices for ecological water quality assessment [WZI (Lougeed and Chow-Fraser 2002), ZBI (De-Carli et al. 2019) and ZRTI (Montagud et al. 2019)] have the drawback of needing the calculation of new optimal/tolerance values (to environmental factors) for the dominant species in order to be applied in different lakes; while P-IBI (Kane et al. 2009), including also phytoplankton data, includes species-specific metrics (i.e. density of *Limnocalanus macrurus*) that should be excluded or adapted for different lakes.

On the other hand, Zoo-IQ development and testing based on the dataset constricted in 13 natural lakes of Greece resulted in the following limitations: a) even though the final metrics have been widely used at higher latitudes (Karabin 1985, Jeppesen et al. 2011, Haberman and Haldna 2014), the final Zoo-IQ index and its boundaries should be tested before applied in different climatic regions (e.g. tropical lakes, temperate lakes, alpine lakes), b) the dataset used covered a wide range of morphological characteristics and the whole trophic spectrum. However, it didn't include Greek lakes with increased salinity due to natural

(e.g. Lake Ismaris, Lake Pikrolimni) or anthropogenic factors (e.g. Lake Koronia). In lakes with increased salinity low abundances can be recorded due to no optimal ranges for zooplankton species which will misindicate high scoring for both A_{Zoo} and B_{Zoo} . Moreover, lakes with increased salinity in the Mediterranean region are characterized by periods of high abundances due to dominance of brachionid rotifers and crustacean communities consist mainly of large bodied calanoids as *Arctopiaptomus salinus* and *Arctodiaptomus spinosus* and daphnids as *Daphnia magna* and *Daphnia mediterranea* [e.g. Spain (Alonso 1990), Cyprus (Karagianni et al. 2018), Greece (Michaloudi and Kostecka 2004, Stamou 2016)]. Thus, in lakes with increased salinity the thresholds Zoo-IQ metrics should be changed based on a dataset consisted only by saline lakes.

The scoring system of the developed index can provide the five-grade classification (high, good, moderate, poor, bad) required by the WFD. However, for an assessment totally compliant with the WFD, further research is needed for the development of ecological quality ratios (EQR) based on reference conditions. In the case of zooplankton communities from Greek lakes there are two sources increasing uncertainty in EQR assessment. These are the lack of undisturbed lakes with minor anthropogenic impacts in the studied lakes and the lack of historical monitoring data (see chapter 3 for details) for modelling purposes (van de Bund and Solimini 2007).

6.6 Conclusions

This is a first attempt to develop a zooplankton-based index for natural lakes of the Mediterranean region. For this zooplankton metrics, representing zooplankton communities' abundance, biomass, structure and size were combined to form the Zooplankton Index of Water Quality (Zoo-IQ). The Zoo-IQ index successfully assessed the water quality of the studied lakes and characterised almost all hypertrophic lakes with poor and deep lakes with good ecological water quality. Moreover, it successfully identified cases with particularities due to fish introductions or implementation of lake water flushing. To this end, Zoo-IQ through the combination of different metrics covering the structure and function of the zooplankton community aims to capture, in an easy and cost-effective way, the complexities of the lake ecosystem. Thus, Zoo-IQ should be used in ecological water quality assessment and restoration monitoring programs of natural Mediterranean lakes.



7. General Discussion

7.1 General Discussion

Zooplankton has a crucial role in the pelagic food web, transferring energy from primary producers to fish (Carpenter et al. 1985, McQueen et al. 1986). Changes in zooplankton abundance and biomass, taxonomic distribution, and size structure can yield information about the state and dynamics of the pelagic ecosystem and food web functioning (Jeppesen et al. 2011). It also responds to the change of the eutrophic status of the water body (e.g. Pejler 1983, Karabin 1985, Stamou et al. 2019a). Thus, zooplankton community structure is an important element in defining the status of the pelagic ecosystem (Caroni and Irvine 2010, Jeppesen et al. 2011). As such it is related directly or indirectly to WFD descriptors of biodiversity, food web function and eutrophication. Thus, despite being left out of the WFD, zooplankton should be considered as a BQE for the ecological water quality assessment.

In the present study we examined zooplankton data of the summer period proposed by the WFD for the ecological status assessment for natural lakes. Mediterranean lakes, which are not well-studied, are differentiated by the well-studied temperate lakes among others [e.g. morphometric and hydrological factors (Alvarez Cobelas et al. 2005)] by biological factors (no biological winter with continuous increase of phytoplankton mainly during late-autumn and winter months and extended reproduction for fish) (Beklioglu et al. 2007, Moustaka-Gouni et al. 2014). Consequently, the food web patterns as the PEG-model (established based on the knowledge of the cold-temperate European lakes) are differentiated in the Mediterranean climatic zones (Moustaka-Gouni et al. 2014).

The studied summer zooplankton communities followed almost the same dominance pattern with mainly rotifers followed by copepods developmental stage, nauplii, dominating in abundance. Biomass was dominated mainly by crustaceans (both copepods and cladocerans), while the dominant species as well as their size were influenced by phytoplankton composition and fish predation. Following these observations, metrics of taxonomic, morphometric and functional traits based on abundance or biomass were selected for the development (or modification) of indices for eutrophication or ecological water quality assessment.

For eutrophication assessment, the trophic state indices based on rotifer TSI_{ROT} (Ejsmont-Karabin 2012) and crustacean communities TSI_{CR} (Ejsmont-Karabin and Karabin 2013), developed for temperate (Polish) lakes, were applied to data from Greek lakes. As it

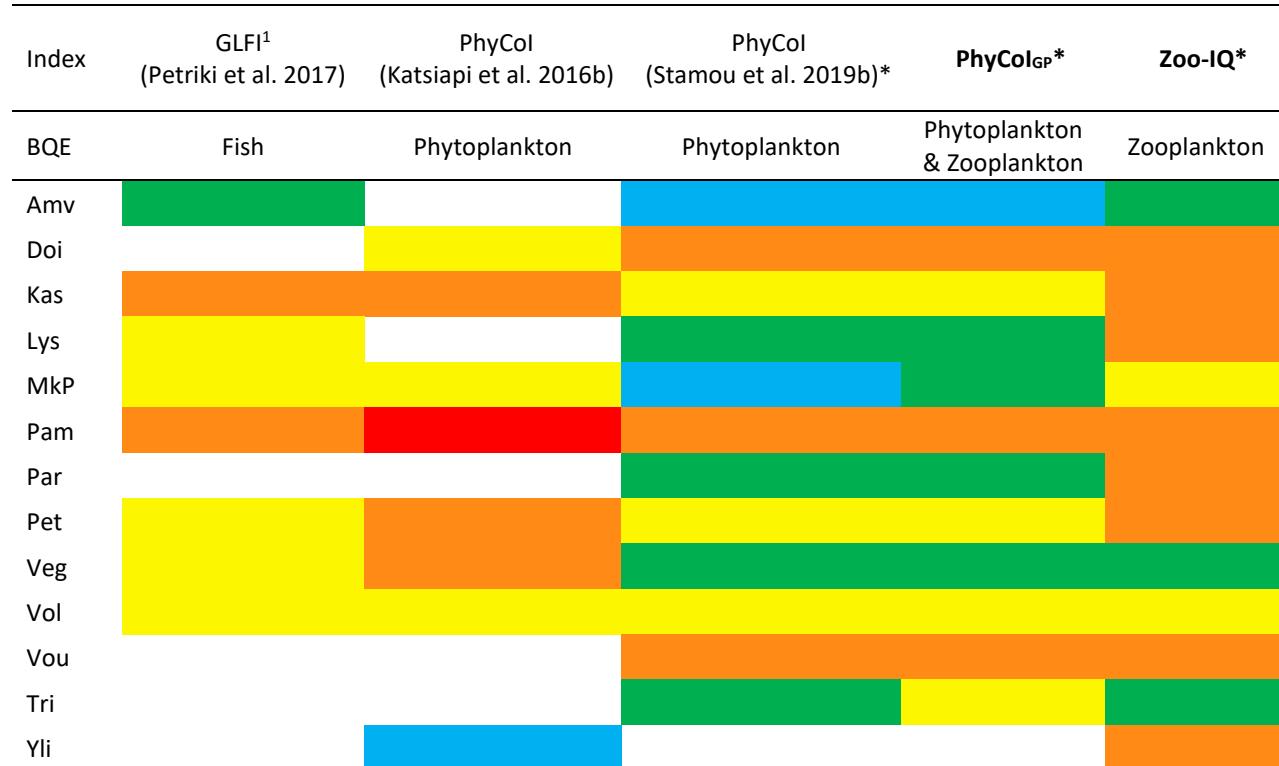
was expected due to the differentiations of plankton communities in the Mediterranean region (Moustaka-Gouni et al. 2014) the two indices did not assess correctly the trophic state, which was estimated based on two eutrophication proxies, TSI_{SD} and the mean summer phytoplankton biomass. Based on the TSI formulae that significantly correlated with the eutrophication proxies, a new index TSI_{Zoo} is proposed combining the whole zooplankton community. In detail, TSI_{Zoo} included two taxonomic metrics based on abundance, [i.e. TSI_{ROT1}: total rotifera abundance (ind/L), TSI_{CR1}: total crustacean (copepoda and cladoceran) abundance (ind/L)] one taxonomic metric based on biomass [TSI_{ROT2}: total wet rotifera biomass (mg/L) excluding *Asplanchna* spp.] and one functional metric [TSI_{CR2}: total wet cyclopoids biomass (mg/L)]. Still, based on the proposed boundaries [by Ejsmont-Karabin (2012) and Ejsmont-Karabin and Karabin (2013)], oligotrophic lakes were overestimated and hypertrophic lakes were underestimated. Hence, there was a need to modify the boundaries, but due to value overlaps this was not possible and further research should be done. However, the zooplankton TSI indices could discriminate two categories of trophic state [i.e low (oligotrophic and mesotrophic lakes) and high (eutrophic and hypertrophic lakes)]. Therefore, TSI_{Zoo} is proposed as a promising index of eutrophication that could be used in monitoring programs of zooplankton communities in the Mediterranean region.

For ecological water quality assessment two indices were developed, PhyCol_{GP} and Zoo-IQ. PhyCol_{GP} is a modification of the phytoplankton based PhyCol index including grazing potential (GP) as a metric. Zooplankton grazing has been indicated as an “additional lake specific - supporting environmental factor featuring the eutrophication impact” (CIS No. 23) (European Commission 2009), trying to compensate for the omission of zooplankton from the WFD. In the present study, the metric GP (%/ day) was selected as a functional metric of the potential zooplankton grazing on phytoplankton (Jeppesen et al. 1997) to enhance the phytoplankton-based ecological status assessment incorporating zooplankton as a supporting factor. The modified PhyCol_{GP} index through the combination of different plankton metrics covering various aspects of the structure and function of the plankton community successfully assessed the water quality of the studied lakes evaluated by PhyCol being more sensitive in some cases. Still, further research is needed in order a plankton index balancing both zooplankton and phytoplankton communities to be developed, incorporating more zooplankton metrics in PhyCol_{GP}.

The Zoo-IQ index is an ecological water quality index based only on data from zooplankton communities. It includes four metrics (A_{Zoo} , B_{Zoo} , MW_{Zoo} and R_{Clad}) equally weighted. Abundance (A_{Zoo}) and biomass (B_{Zoo}) of the whole zooplankton community (rotifers, cladocerans and copepods) which are known to increase across eutrophication gradient (e.g. Karabin 1985), mean zooplankton size (MW_{Zoo}) as a morphometric trait of the size structure of the community and the ratio of large cladocerans to total cladocerans abundance (R_{Clad}) influenced both by eutrophication and fish predation (Jeppesen et al. 2011). The Zoo-IQ index successfully assessed the water quality of the studied lakes and characterised almost all hypertrophic lakes with poor and deep lakes with good ecological water quality. Moreover, it successfully identified cases being under the implementation of restoration/biomanipulation measures.

In the present study, the ecological water quality status was identified using PhyColGP and Zoo-IQ indices. Differences in the status of the same year was found for lakes Amvrakia, Kastoria, Mikri Prespa, Paralimni and Trichonis (Table 7.1). This is expected because each biological element responds differently to the pressure exerted and such differences are more pronounced when indices based on various BQEs are used (Lyche-Solheim et al. 2013). In the case of Greek lakes indices based on all pelagic BQEs (fish, phytoplankton, zooplankton) have been developed and their application is presented in Table 7.1, using colour interpretation in accordance with the requirements of WFD (i.e. high-blue, good-green, moderate-yellow, poor-orange and poor-red). The comparison of the ecological water quality should take into consideration that each index was applied in different years, influenced by different environmental or anthropogenic factors. This is even more evident when the same index is applied in different years; PhyCol index applied for different years of the studied lakes in Katsiapi et al. (2016b) and Stamou et al. (2019b) resulted in different assessments. Nevertheless, the water quality assessment based on PhyCol and PhyCol_{GP} of lakes Amvrakia (characterised as high) and Trichonis (characterised as good by PhyCol and moderate by PhyCol_{GP}) raises another issue when taking into consideration that both lakes exhibited pronounced deep maxima of cyanobacteria species (Moustaka-Gouni et al. 2019a), while in the case of Lake Trichonis cyanotoxins have also been detected both in the shallow and deep waters (Christophoridis et al. 2018). This indicates that in such cases phytoplankton samples should cover the whole water column and not just the euphotic zone used by several countries implementing the WFD (Moustaka-Gouni et al. 2019a).

*Table 7.1 Ecological water quality of the 13 natural Greek lake based on different BQEs of the pelagic zone. Lakes abbreviations according to Table 2.1. Indices in bold were developed in the present study, ¹ GLFI is the only index estimating water quality based on EQR and * indicates the same study period*



Generally, during the 20 years of the implementation of the WFD, many studies have been published based on the achievements and the drawbacks of the WFD in various countries (e.g. Ietswaart 2006, Moss et al. 2008, Nõges et al. 2009, Prato et al. 2014, Poikane et al. 2015, Voulvouli et al. 2017, Moustaka-Gouni et al. 2019b) in order to discuss critical issues and practices, and recommend approaches to improve the implementation process in the future. Two of the critical issues are the "one-out, all-out" principle and the typology system. It has been found that in some cases the "one-out-all-out" principle tends to downgrade sites unjustifiably (Moss et al. 2003, Nõges et al. 2009, Prato et al. 2014). In order to avoid such errors, three practices have been recommended, a) the use of multi-metric indices with equal number of metrics per various BQEs, b) the increase of sampling frequency or density, to reduce the variation in each element (not always possible, due to financial constraints) and c) the omission of elements with high and not biologically explained variability from the assessment (Moss et al. 2003, Borja and Rodríguez 2010). Still, this indicates the need for alternative approaches to the one-out, all-out" principle. As for the WFD typology schemes, they have often been criticized, especially due to identification of

>650 national lake types not corresponding with the common intercalibration types (Poikane et al. 2014, Lyche Solheim et al. 2015). In the case of Greece, a constructive critique to help legal authorities to better understand and revise the national typology scheme of the Greek lakes has been recently published (Moustaka-Gouni et al. 2019b). The national typology of Greek lakes has failed to take into account essential components such as lake size, altitude, salinity, climate factors, and inherent detritus water content. Lately, a new broad typology scheme for European lakes has been developed reflecting the natural variability in type descriptors (Lyche Solheim et al. 2019). Thus, more improvements in WFD implementation are expected in the following years.

As articulated in the Directive definitions (Article 2), ecological status is an expression of the quality of the structure and functioning of aquatic ecosystems (European Commission 2000). However, functional metrics are rarely included in the indices used by the Member States for the ecological water quality assessment according to the WFD (Poikane et al. 2015). According to Moustaka-Gouni et al. (2019b) recommendations a future index development should be based both on structural and functional attributes of the communities such as food-web metrics. Future studies should focus on the development of a multimetric index based on the principal components of the food webs (e.g. pelagic food web: phytoplankton, zooplankton and fish), equally weighted even combined with physico-chemical elements, in order to define quality in an integrative way. Such an index will permit the assessment of ecological status using an ecosystem/holistic approach.

In conclusion, based on the findings of the present study, zooplankton has a strong indicator value, when selecting the right metrics covering taxonomic, morphometric and functional diversity. Therefore, zooplankton is strongly recommended as a central BQE that should be included in the WFD assessments and undertake similar calibration exercises to obtain relevant and robust metrics as is the case for other BQEs. In the present study three indices were developed/modified (i.e. TSI_{Zoo}, PhyCol_{GP} and Zoo-IQ) based on data from Greek lakes. All three indices are cost-effective, since zooplankton has relatively cheap and not time-consuming samplings and easy to measure since they don't need identification down to species level. Moreover, they successfully assessed the trophic state or ecological water quality when applied to data from Greek lakes. Therefore, the use of the index TSI_{Zoo} is proposed for the eutrophication assessment and the indices PhyCol_{GP} (combining also

7.1 General Discussion

phytoplankton data) and Zoo-IQ are proposed for ecological water quality assessment of natural lakes in the Mediterranean region.



8. Conclusions

8.1 Conclusions

Summer zooplankton communities

- The new data of the present study (summer 2016-2017) contributed to the existing knowledge of the zooplankton communities with new data for lakes Amvrakia, Kastoria, Lysimachia, Ozeros, Pamvotis, Trichonis, Vegeritis and Voulkaria.
- Data for the zooplankton communities of the Lakes Kremasta and Paralimni were recorded for the first time.
- The studied summer zooplankton communities followed almost the same dominance pattern with mainly rotifers followed by copepods developmental stage, nauplii dominating in abundance.
- Biomass was dominated mainly by crustaceans (both copepods and cladocerans). The dominant species were influenced by phytoplankton composition (e.g. chydorids and bosminids in hypertrophic lakes dominated by cyanobacteria).
- Lake Ozeros had a different pattern of dominance, since it was the only lake with a high dominance of mollusc larvae (over 60% contribution)
- During summer a shift in biomass dominance was recorded, from larger crustacean species to a) rotifers and b) smaller cladoceran species.
- Dominance of large-bodied individuals over 50% was recorded only in lakes Amvrakia, Kastoria, Tavropos, Trichonis and Vegeritis mainly due to adult copepods and large cladocerans, indicating increased fish predation in the Mediterranean lakes.
- The studied lakes were clustered into six groups based on their copepod communities, regardless of the lake's trophic state or morphological characteristics.
- The dominance of the main groups and their size can provide information related to the lakes' trophic characteristics and food web function.

Trophic State Indices

- The trophic state indices TSI_{ROT} and TSI_{CR} were significantly correlated with the eutrophication proxies TSI_{SD} and phytoplankton biomass.
- TSI_{ROT} and TSI_{CR} did not successfully assess the trophic state underestimating the hypertrophic lakes and overestimating the oligotrophic lakes indicating the need to

modify the proposed boundaries. However, due to range overlaps this needs further investigation.

- A new index namely TSI_{zoo} (Zooplankton Trophic State Index), was developed as the average of only the formulae (TSI_{ROT1}, TSI_{ROT2}, TSI_{CR1} and TSI_{CR2}) that significantly correlated with both eutrophication proxies.
- TSI_{zoo} is the index best correlated with both eutrophication proxies and thus, the most efficient in discriminating eutrophication.
- TSI_{zoo}, TSI_{ROT} and TSI_{CR} can detect efficiently two groups of low (oligotrophic-mesotrophic) and high (eutrophic-hypertrophic) trophic state when applied in the Mediterranean region using the boundaries for TSI_{ZOO} and TSI_{ROT} <45 and TSI_{CR} <50 for low trophic state.
- TSI_{zoo} is proposed as a promising, effective and cost-effective tool for large scale and long-term monitoring programs of Mediterranean lakes.

Grazing Potential

- Grazing Potential (GP) index applied on plankton communities of natural lakes during the summer period is efficient in discriminating ecological water quality based on the functional traits of both plankton communities.
- Lakes with high GP values have high zooplankton biomass dominated by large cladocerans or/and calanoids
- Lakes with low GP values are characterized by increased phytoplankton biomass and/or by small bodied zooplankton indicating intensive fish predation.
- GP was included in a modified version of the phytoplankton-based index PhyCol, namely PhyCol_{GP} index.
- The PhyCol_{GP} successfully assessed the water quality of the studied lakes evaluated by PhyCol appearing more sensitive in cases under restoration measures.
- PhyCol_{GP} should be used in other climatic zones using the appropriate classification scheme for the metric TB (total biovolume).
- PhyCol_{GP} is proposed to be used in a cost-effective monitoring for natural Mediterranean lakes using high frequency Secchi Depth measurements combined with low frequency of PhyCol_{GP} determinations, especially during the warm period.

Zooplanktonic-Index of Water Quality

- The Zoo-IQ index, an ecological water quality index, was developed based only on zooplankton data including four metrics [abundance (A_{Zoo}), biomass (B_{Zoo}), mean weight (MW_{Zoo}) and ratio of large cladocerans to total cladocerans (R_{Clad})] equally weighted, providing a five-grade classification scheme.
- Zoo-IQ index successfully assessed the ecological water quality of the Greek lakes since almost all hypertrophic lakes were characterised as poor and deep lakes as good and it identified cases with particularities due to the implementation of restoration programs.
- The scoring system of the Zoo-IQ should be adapted in order to be applied in lakes with increased salinity in the Mediterranean region and for all type of lakes in different climatic zones.
- Zoo-IQ index is proposed as an easy to use and effective index for monitoring purposes during the implementation of restoration plans to prevent further deterioration.

General Conclusions

- Zooplankton proved to be an effective element for the assessment and monitoring of both eutrophication and ecological water quality
- The inclusion of zooplankton as a biological quality element in the Water Framework Directive for the ecological water quality assessment is strongly proposed.
- Future studies should focus on the development of a multimetric index based on the pelagic biological elements of the food webs (e.g. pelagic food web: phytoplankton, zooplankton and fish) in order to define quality in an ecosystem approach.



9. References

9. References

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Supplementary Material

Appendix 1 Publications of the period 2000-2019 including zooplankton community data from Greek lakes included in the national monitoring program

Reference	Sampling date	Lakes
Antonopoulos et al. 2008	2004-2005	Pamvotis
Chalkia and Kehayias 2013a	2009-2010	Ozeros
Chalkia and Kehayias 2013b	2009-2010	Lysimachia
Chalkia et al. 2012	2006-2008	Amvrakia
Doulka and Kehayias 2008	2003-2005	Trichonis
Doulka et al. 2013	2006-2007	Trichonis
Doulka and Kehayias 2011	2005	Trichonis
Kagalou et al. 2003	1998-9999	Pamvotis
Kagalou et al. 2009	different for each lake	Amvrakia, Cheimaditis, Doirani, Kastoria, Koronia, Lysimachia, Megali Prespa, Mikri Prespa, Ozeros, Pamvotida, Petron, Trichonis, Vegoritis, Volvi, Zazari
Katsiapi et al. 2012	2008	Megali Prespa
Kehayias et al. 2004	2002-2003	Trichonis
Kehayias et al. 2008	2004-2006	Stratos
Kehayias et al. 2018	2014	Trichonis
Kleanthidis and Sinis 2001	1995-1996	Volvi
Marrone et al. 2019 ¹		Kourna
Matzafleri et al. 2017	2001	Kastoria
Mazaris et al. 2010	different for each lake	Doirani, Kastoria, Mikri Prespa, Tavropos, Vegoritis, Volvi, Yliki
Michaloudi and Kostecka 2004	1999-2000	Koronia
Michaloudi 2005	1990-1992	Mikri Prespa
Michaloudi et al. 2009	2004	Koronia
Michaloudi et al. 2012	2003-2004	Koronia
Moustaka-Gouni et al. 2006	1999	Kastoria
Moustaka-Gouni et al. 2012	2003-2004, 2005-2007, 2009-2010	Koronia
Moustaka-Gouni et al. 2014	different for each lake	Kastoria, Mikri Prespa, Tavropos, Vegoritis, Volvi and Yliki
Stamou et al. 2017 ²	different for each lake	Amvrakia, Cheimaditis, Doirani, Doxa-Feneou, Ismaris, Karla, Kastoria, Koronia, Kourna, Ladona, Lysimachia, Megali Prespa, Mikri Prespa, Ozeros, Pamvotida, Petron, Pikrolimni, Piniou, Stymfalia, Tavropos Trichonis, Vegoritis, Volvi, Vouklaria, Yliki, Zazari
Stefanidis and Papastergiadou 2010	2006-2008	Kastoria, Mikri Prespa, Petron, Vegoritis

¹ Only data from copepods community

² Only data from rotifers communities

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Supplementary Material

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Appendix 2 Taxa's contribution (%) to total abundance of zooplankton communities from the 17 Greek lakes. Yellow colour indicates the dominating (> 20%) taxa. Codes according to samplings abbreviation in Figure 3.1

Codes	Amv_Jl_16	Amv_Au_16	Amv_Se_16	Doi_Jl_04	Doi_Au_04	Doi_Se_04	Kas_Jl_99	Kas_Au_99	Kas_Se_99	Kas_Jl_16	Kas_Au_16	Kas_Se_16	Kre_Jl_16	Kre_Au_16	Kre_Se_16	Lys_Jl_16	Lys_Au_16	Lys_Se_16	MgP_Jn_16	MgP_Jl_16	MgP_Au_16	MgP_Jn_90	MkP_Jl_90	MkP_Au_90	
Bivalvia	25	20	18	0	0	0	0	0	0	0	0	0	10	41	20	0	0	0	0	0	0	0	0	0	0
Rotifera																									
<i>Anuraeopsis fissa</i> (Gosse, 1851)	0	0	0	0	0	0	1	19	1	2	0	21	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha ecaudis</i> Perty, 1850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha ovalis</i> (Bergendal, 1892)	0	0	0	0	0	0	0	0	0	0	0	0	1	0	7	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha sultans</i> Bartsch, 1870	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna girodi</i> Guerne, 1888	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna priodonta</i> Gosse, 1850	1	0	0	0	0	0	0	0	0	0	0	0	4	1	1	0	0	0	0	0	0	0	0	1	0
<i>Asplanchna</i> spp. Gosse, 1850	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bdelloidea</i> Hudson, 1884	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus angularis</i> Gosse, 1851	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	22
<i>Brachionus budapestinensis</i> Daday, 1885	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus calyciflorus</i> Pallas, 1766	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0	2	3	0	0	0	0	0	0	0	0
<i>Brachionus cf. quadridentatus</i> Hermann, 1783	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus dimidiatus</i> Bryce, 1931	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus diversicornis</i> (Daday, 1883)	0	0	0	8	1	11	3	4	2	0	15	2	0	0	0	2	2	0	0	0	0	0	0	1	2
<i>Brachionus forficula</i> Wierzejski, 1891	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus ibericus</i> Ciros-Pérez, Gómez & Serra, 2001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus sessilis</i> Varga, 1951	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus urceolaris</i> Müller, 1773	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cephalodella gibba</i> (Ehrenberg, 1830)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cephalodella</i> sp. Bory de St. Vincent, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Collotheca</i> sp. Harring, 1913	2	2	0	0	0	0	0	1	1	0	5	3	0	0	2	0	0	0	1	0	0	0	2	1	
<i>Conochilus</i> sp. Ehrenberg, 1834	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conochilus dossuarius</i> Hudson, 1885	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conochilus unicornis</i> Rousselet, 1892	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epiphantes macroura</i> (Barrois & Daday, 1894)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euchlanis dilatata</i> Ehrenberg, 1830	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Filinia longisetata</i> (Ehrenberg, 1834)	0	0	0	0	3	1	11	5	0	2	21	0	0	0	1	0	0	0	1	9	8	0	0	0	0
<i>Filinia opoliensis</i> (Zacharias, 1898)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	37	10	23	0	0	0	0	0	0	0	0
<i>Filinia terminalis</i> (Plate, 1886)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gastropus stylifer</i> Imhof, 1891	0	0	0	0	0	0	0	0	0	0	0	0	5	1	0	0	0	0	0	0	0	0	0	0	0
<i>Hexarthra bulgarica</i> (Wiszniewski, 1933)	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexarthra mira</i> (Hudson, 1871)	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexarthra</i> sp. Schmarda, 1854	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Kellicottia longispina</i> (Kellicott, 1879)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Keratella cochlearis</i> (Gosse, 1851)	0	0	0	3	0	4	5	9	17	29	1	18	2	0	0	1	17	12	40	8	1	4	9	1	
<i>Keratella quadrata</i> (Müller, 1786)	0	0	0	1	1	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Keratella tecta</i> (Gosse, 1851)	0	0	0	25	1	11	0	3	1	1	1	6	0	0	0	0	0	3	0	0	0	0	0	0	0

Codes	Amv_Jl_16	Amv_Au_16	Amv_Se_16	Doi_Jl_04	Doi_Au_04	Doi_Se_04	Kas_Jl_99	Kas_Au_99	Kas_Se_99	Kas_Jl_16	Kas_Au_16	Kas_Se_16	Kre_Jl_16	Kre_Au_16	Kre_Se_16	Lys_Jl_16	Lys_Au_16	Lys_Se_16	Mgp_Jn_16	Mgp_Jl_16	Mgp_Au_16	Mgp_Jn_90	Mgp_Jl_90	Mkp_Au_90	Mkp_Jl_90	Mkp_Jn_90
<i>Keratella tropica</i> (Apstein, 1907)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane bulla</i> (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane closterocerca</i> (Schmarda, 1859)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane luna</i> (Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane quadridentata</i> (Ehrenberg, 1830)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella ehrenbergii</i> (Perty, 1850)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella patella</i> (Müller, 1773)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella</i> sp. Bory de St. Vincent, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Monomma actices</i> Myers, 1930	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ploesoma hudsoni</i> (Imhof, 1891)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ploesoma truncatum</i> (Levander, 1894)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0
<i>Polyarthra dolichoptera</i> Idelson, 1925	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	17	17	0	0	0	0	0	0
<i>Polyarthra luminosa</i> Kutikova, 1962	1	0	0	0	0	0	0	0	0	0	0	0	0	6	11	33	0	0	0	0	0	0	0	0	0	0
<i>Polyarthra</i> sp. Ehrenberg, 1834	0	0	0	17	35	32	12	9	37	36	26	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polyarthra vulgaris</i> Carlin, 1943	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	1	7	0	0	0	0
<i>Pompholyx sulcata</i> Hudson, 1885	3	2	0	19	0	1	0	1	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Proalides subtilis</i> Rodewald, 1940	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Scardium longicaudum</i> (Müller, 1786)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Squatinella lamellaris</i> (Müller, 1786)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Synchaeta</i> sp. Ehrenberg, 1832	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	1	0	0	0	0	0	0	0	0	0	0
<i>Synchaeta stylata</i> Wierzejski, 1893	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0
<i>Trichocerca capucina</i> (Wierzejski & Zacharias, 1893)	0	0	0	0	0	1	3	2	2	1	1	1	0	0	0	0	0	0	0	0	0	7	2	7	2	
<i>Trichocerca cylindrica</i> (Imhof, 1891)	0	0	0	0	0	0	1	7	8	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca dixonnuttalli</i> (Jennings, 1903)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca pusilla</i> (Jennings, 1903)	0	0	0	0	1	0	0	1	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca ruttneri</i> Donner, 1953	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca similis</i> (Wierzejski, 1893)	0	0	0	12	6	3	21	10	0	1	27	6	1	1	1	14	17	1	5	8	1	2	1	2		
<i>Trichocerca</i> sp. Lamarck, 1801 ²	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unidentified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladocera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bosmina (Bosmina) longirostris</i> (O. F. Müller, 1776)	1	3	0	0	0	0	2	0	0	0	0	0	6	4	2	13	4	3	0	0	0	0	0	0	0	0
<i>Ceriodaphnia dubia</i> Richard, 1894	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceriodaphnia pulchella</i> Sars, 1862	2	2	11	0	0	0	4	0	0	3	0	0	0	0	0	1	1	0	0	0	0	0	7	4	0	0
<i>Ceriodaphnia quadrangula</i> (O. F. Müller, 1785)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chydorus sphaericus</i> (O. F. Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Coronatella rectangula</i> (Sars, 1862)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Daphnia (Daphnia) cucullata</i> Sars, 1862	2	2	1	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	2	2	0	47	10	1		
<i>Daphnia (Daphnia) galeata</i> Sars, 1864	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Daphnia</i> spp. O. F. Mueller, 1785	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diaphanosoma</i> spp. Fischer, 1850	1	16	7	2	7	1	8	0	0	0	0	0	6	4	3	9	1	1	4	13	25	4	9	7		
<i>Macrothrix hirsuticornis</i> Norman & Brady, 1867	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Moina micrura</i> Kurz, 1875	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
<i>Leptodora kidtnii</i> (Focke, 1844)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 2 Continued

Codes	Amv_Jl_16	Amv_Au_16	Amv_Se_16	DoI_Jl_04	DoI_Au_04	DoI_Se_04	Kas_Jl_99	Kas_Au_99	Kas_Se_99	Oze_Jl_16	Oze_Au_16	Oze_Se_16	Pam_Jl_16	Pam_Au_16	Pam_Se_16	Par_Jl_17	Par_Au_17	Par_Se_17	Pet_Jl_10	Pet_Au_10	Pet_Se_10	Tau_Jl_90	Tau_Au_87	Tau_Se_87	MkP_Jl_16	MkP_Au_16	Mgp_Se_16	Mgp_Jn_16	Mgp_Au_16	Mgp_Se_16	MkP_Jl_90	MkP_Au_90	MkP_Se_90		
Nauplii	12	19	27	10	32	13	21	14	11	2	11	4	40	30	28	14	16	32	13	15	18	19	5	2	3										
Copepodite Calanoida	33	12	16	0	0	0	0	0	0	0	0	0	0	0	0	2	2	1	4	1	5	4	4	12	14										
Copepodite Cyclopoida	2	2	2	1	11	7	8	6	1	8	4	1	4	2	2	2	1	4	0	0	0	0	0	0	0	0	0	0	0						
<i>Acanthocyclops robustus</i> group (Sars G.O., 1863)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Arctodiaptomus steindachneri</i> (Richard, 1897)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Cyclops vicinus vicinus</i> Ulianin, 1875	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Eucyclops serrulatus serrulatus</i> (Fischer, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Eudiaptomus drieschi</i> (Poppe & Mrázek, 1895)	13	16	14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Eudiaptomus gracilis</i> (Sars G.O., 1863)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Macrocyclops albidus albidus</i> (Jurine, 1820)	2	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Mesocyclops leuckarti leuckarti</i> (Claus, 1857)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Neolovenula alluaudi</i> (Guerne & Richard, 1890)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Thermocyclops</i> spp. Kiefer, 1927	0	0	0	0	1	1	1	0	3	3	3	1	3	2	0	1	3	1	0	0	0	0	0	0	0	0	0	0	0	0					
Cyclopoida unidentified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
 Codes	 MkP_Jl_91	 MkP_Au_91	 MkP_Se_91	 MkP_Jl_92	 MkP_Au_92	 MkP_Se_92	 MkP_Jn_16	 MkP_Au_16	 MkP_Se_16	 Oze_Jl_16	 Oze_Au_16	 Oze_Se_16	 Pam_Jl_16	 Pam_Au_16	 Pam_Se_16	 Par_Jl_17	 Par_Au_17	 Par_Se_17	 Pet_Jl_10	 Pet_Au_10	 Pet_Se_10	 Tau_Jl_90	 Tau_Au_87	 Tau_Se_87	 Mgp_Jn_16	 Mgp_Au_16	 Mgp_Se_16	 MkP_Jl_90	 MkP_Au_90	 MkP_Se_90					
 Bivalvia	 0	 68	 56	 0	 0	 0	 1	 3	 0	 0	 0	 0	 0	 0																					
 Rotifera	 0	 0	 0	 0	 0																														
<i>Anuraeopsis fissa</i> (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Ascomorpha ecaudis</i> Perty, 1850	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Ascomorpha ovalis</i> (Bergental, 1892)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Ascomorpha saltans</i> Bartsch, 1870	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Asplanchna girodi</i> Guerne, 1888	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Asplanchna priodonta</i> Gosse, 1850	0	0	0	0	1	0	0	0	0	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Asplanchna</i> spp. Gosse, 1850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
Bdelloidea Hudson, 1884	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Brachionus angularis</i> Gosse, 1851	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Brachionus budapestinensis</i> Daday, 1885	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Brachionus calyciflorus</i> Pallas, 1766	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Brachionus cf. quadridentatus</i> Hermann, 1783	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Brachionus dimidiatus</i> Bryce, 1931	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Brachionus diversicornis</i> (Daday, 1883)	0	0	3	3	1	1	1	0	0	0	0	0	0	0	9	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Brachionus forcipula</i> Wierzejski, 1891	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Brachionus ibericus</i> Ciros-Pérez, Gómez & Serra, 2001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Brachionus sessilis</i> Varga, 1951	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Brachionus urceolaris</i> Müller, 1773	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Cephalodella gibba</i> (Ehrenberg, 1830)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Cephalodella</i> sp. Bory de St. Vincent, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Collotheca</i> sp. Herring, 1913	0	0	0	0	0	0	0	0	0	0	3	0	0	0	6	0	0	4	1	1	0	0	0	0	0	0	0	0	0	0					
<i>Conochilus</i> sp. Ehrenberg, 1834	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0					
<i>Conochilus dosvarius</i> Hudson, 1885	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	7	6	0	0	0	0	0	0	0	0	0					

Codes	MkP_Jl_91	MkP_Au_91	MkP_Se_91	MkP_Jl_92	MkP_Au_92	MkP_Se_92	MkP_Jn_16	MkP_Jl_16	MkP_Au_16	Oze_Jl_16	Oze_Au_16	Oze_Se_16	Pam_Jl_16	Pam_Au_16	Pam_Se_16	Par_Jl_17	Par_Au_17	Par_Se_17	Par_Jl_10	Pet_Au_10	Pet_Se_10	Tau_Jl_87	Tau_Au_87	Tau_Se_87	
<i>Conochilus unicornis</i> Rousselet, 1892	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epiphantes macroura</i> (Barrois & Daday, 1894)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euchlanis dilatata</i> Ehrenberg, 1830	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Filinia longisetosa</i> (Ehrenberg, 1834)	69	3	2	3	0	0	3	0	0	0	0	7	0	9	0	0	0	12	8	36	0	0	0	0	0
<i>Filinia opoliensis</i> (Zacharias, 1898)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Filinia terminalis</i> (Plate, 1886)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gastropus stylifer</i> Imhof, 1891	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexarthra bulgarica</i> (Wiszniewski, 1933)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexarthra mira</i> (Hudson, 1871)	0	0	0	0	0	0	0	0	5	7	8	4	4	25	8	11	7	0	0	0	0	0	0	0	0
<i>Hexarthra</i> sp. Schmarda, 1854	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Kellicottia longispina</i> (Kellicott, 1879)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	9	16
<i>Keratella cochlearis</i> (Gosse, 1851)	0	1	5	4	1	0	38	12	30	0	0	0	14	0	0	13	2	1	0	0	0	1	0	4	0
<i>Keratella quadrata</i> (Müller, 1786)	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Keratella tecta</i> (Gosse, 1851)	0	0	0	0	0	0	0	2	1	0	0	0	0	0	1	0	13	1	0	0	1	0	0	0	0
<i>Keratella tropica</i> (Apstein, 1907)	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Lecane bulla</i> (Gosse, 1851)	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane closterocerca</i> (Schmarda, 1859)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane luna</i> (Müller, 1776)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane quadridentata</i> (Ehrenberg, 1830)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella ehrenbergii</i> (Perty, 1850)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella patella</i> (Müller, 1773)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella</i> sp. Bory de St. Vincent, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Monommaea actices</i> Myers, 1930	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ploesoma hudsoni</i> (Imhof, 1891)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ploesoma truncatum</i> (Levander, 1894)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polyarthra dolichoptera</i> Idelson, 1925	0	0	0	0	0	0	0	0	0	1	16	18	6	4	0	0	0	0	0	0	0	0	0	0	0
<i>Polyarthra luminosa</i> Kutikova, 1962	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polyarthra</i> sp. Ehrenberg, 1834	1	1	0	1	4	0	0	0	0	0	0	0	0	0	0	0	23	52	0	5	20	35	0	0	11
<i>Polyarthra vulgaris</i> Carlin, 1943	0	0	0	0	0	0	0	7	3	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pompholyx sulcata</i> Hudson, 1885	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	0	0	0	0	0	0	0
<i>Proalides subtilis</i> Rodewald, 1940	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Scarium longicaudum</i> (Müller, 1786)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Squatinella lamellaris</i> (Müller, 1786)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Synchaeta</i> sp. Ehrenberg, 1832	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	53	0	0	0	0	0	0
<i>Synchaeta stylata</i> Wierzejski, 1893	0	0	0	0	0	0	0	0	0	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca capucina</i> (Wierzejski & Zacharias, 1893)	1	1	2	1	5	7	5	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca cylindrica</i> (Imhof, 1891)	0	0	34	0	0	0	1	0	0	0	0	5	1	1	0	2	1	0	0	0	0	0	0	0	0
<i>Trichocerca dixonnuttalli</i> (Jennings, 1903)	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca pusilla</i> (Jennings, 1903)	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Trichocerca ruttneri</i> Donner, 1953	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca similis</i> (Wierzejski, 1893)	4	2	6	2	2	1	3	9	6	31	0	3	17	12	0	1	0	0	0	0	0	0	0	2	
<i>Trichocerca</i> sp. Lamarck, 1801 ²	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Unidentified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladocera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 2 Continued

Codes	Tri_Jl_16	Tri_Au_16	Tri_Se_16	Veg_Jl_87	Veg_Au_87	Veg_Se_87	Veg_Jn_17	Veg_Au_17	Veg_Se_17	Veg_Jl_84	Vol_Au_84	Vol_Se_84	Vol_Jl_85	Vol_Au_85	Vol_Se_85	Vol_Jn_86	Vol_Au_86	Vol_Se_86	Vou_Jl_16	Vou_Au_16	Vou_Se_16	Vou_Jl_90	Vou_Au_90	Vou_Se_90	
Bivalvia	7	32	33	0	0	0	38	33	9	1	2	0	0	4	4	5	0	0	0	0	0	0	0	0	0
Rotifera																									
<i>Anuraeopsis fissa</i> (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Ascomorpha ecaudis</i> Perty, 1850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	2
<i>Ascomorpha ovalis</i> (Bergendal, 1892)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha saltans</i> Bartsch, 1870	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna girodi</i> Guerne, 1888	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna priodonta</i> Gosse, 1850	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
<i>Asplanchna</i> spp. Gosse, 1850	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bdelloidea</i> Hudson, 1884	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus angularis</i> Gosse, 1851	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosmina (Bosmina) longirostris (O. F. Müller, 1776)	0	0	0	0	0	0	0	0	0	1	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceriodaphnia dubia</i> Richard, 1894	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceriodaphnia pulchella</i> Sars, 1862	0	20	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceriodaphnia quadrangula</i> (O. F. Müller, 1785)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chydorus sphaericus</i> (O. F. Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Coronatella rectangula</i> (Sars, 1862)	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Daphnia (Daphnia) cucullata</i> Sars, 1862	2	4	3	10	8	2	0	0	0	0	0	0	0	7	5	22	0	0	0	0	0	0	0	0	0
<i>Daphnia (Daphnia) galeata</i> Sars, 1864	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Daphnia</i> spp. O. F. Mueller, 1785	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32	27	23
<i>Diaphanosoma</i> spp. Fischer, 1850	0	0	6	15	30	22	0	1	3	11	1	2	6	1	8	6	0	1	13	7	0	37	22	4	
<i>Macrothrix hirsuticornis</i> Norman & Brady, 1867	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Moina micrura</i> Kurz, 1875	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Leptodora kidtnii</i> (Focke, 1844)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nauplii	14	37	21	25	32	40	20	46	7	34	2	4	13	13	9	13	12	12	25	11	10	6	9	4	
Copepodite Calanoida	1	8	4	10	6	12	8	7	6	12	1	1	1	1	3	0	0	0	0	0	0	0	0	0	
Copepodite Cyclopoida	4	13	9	10	3	6	2	3	12	0	0	0	0	5	3	8	8	2	13	2	17	5	4	10	19
<i>Acanthocyclops robustus</i> group (Sars G.O., 1863)	0	0	0	0	0	0	0	0	0	0	0	0	1	2	1	0	0	0	1	1	0	0	0	0	0
<i>Arctodiaptomus steindachneri</i> (Richard, 1897)	3	3	2	9	4	4	3	9	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclops vicinus vicinus</i> Ulljanin, 1875	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	
<i>Eucyclops serrulatus serrulatus</i> (Fischer, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eudiaptomus drieschi</i> (Poppe & Mrázeck, 1895)	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eudiaptomus gracilis</i> (Sars G.O., 1863)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Macrocyclops albidus albidus</i> (Jurine, 1820)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mesocyclops leuckarti leuckarti</i> (Claus, 1857)	1	4	3	5	3	4	3	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neolovenula alluaudi</i> (Guerne & Richard, 1890)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Thermocyclops</i> spp. Kiefer, 1927	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	1	0	0	0	0	0	0	0	0
Cyclopoida unidentified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	4	4	4

Codes	Tri_Jl_16	Tri_Au_16	Tri_Se_16	Veg_Jl_87	Veg_Au_87	Veg_Se_87	Veg_Jn_17	Veg_Au_17	Veg_Se_17	Veg_Jl_84	Vol_Au_84	Vol_Se_84	Vol_Jl_85	Vol_Au_85	Vol_Se_85	Vol_Jn_86	Vol_Au_86	Vol_Se_86	Vou_Jl_16	Vou_Au_16	Vou_Se_16	Vou_Jl_90	Vou_Au_90	Vou_Se_90	
Bivalvia	7	32	33	0	0	0	38	33	9	1	2	0	0	0	4	4	5	0	0	0	0	0	0	0	0
Rotifera																									
<i>Anuraeopsis fissa</i> (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Ascomorpha ecaudis</i> Perty, 1850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	2	
<i>Ascomorpha ovalis</i> (Bergendal, 1892)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha saltans</i> Bartsch, 1870	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna girodi</i> Guerne, 1888	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna priodonta</i> Gosse, 1850	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
<i>Asplanchna</i> spp. Gosse, 1850	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bdelloidea</i> Hudson, 1884	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus angularis</i> Gosse, 1851	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 2 Continued

Codes	Tri_Jl_16	Tri_Au_16	Tri_Se_16	Veg_Jl_87	Veg_Au_87	Veg_Se_87	Veg_Jn_17	Veg_Jl_84	Veg_Au_84	Veg_Se_84	Vol_Jl_85	Vol_Au_85	Vol_Se_85	Vol_In_86	Vol_Jl_86	Vol_Au_86	Vol_Se_16	Vol_Au_16	Vol_Se_16	Yi_Jl_90	Yi_Au_90	Yi_Se_90	
Brachionus budapestinensis Daday, 1885	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	34	1	0	0	0	0	0	
Brachionus calyciflorus Pallas, 1766	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Brachionus cf. quadridentatus Hermann, 1783	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Brachionus dimidiatus Bryce, 1931	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Brachionus diversicornis (Daday, 1883)	0	0	0	0	0	0	0	0	0	33	11	4	3	1	0	0	1	0	0	2	6	0	
Brachionus forficula Wierzejski, 1891	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Brachionus ibericus Ciros-Pérez, Gómez & Serra, 2001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Brachionus sessilis Varga, 1951	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Brachionus urceolaris Müller, 1773	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Cephalodella gibba (Ehrenberg, 1830)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Cephalodella sp. Bory de St. Vincent, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Collotheca sp. Herring, 1913	1	1	1	0	0	2	0	0	0	0	0	0	0	2	2	0	0	2	7	0	3	0	0
Conochilus sp. Ehrenberg, 1834	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Conochilus dossuarius Hudson, 1885	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Conochilus unicornis Rousset, 1892	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Epiphanea macroura (Barrois & Daday, 1894)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	
Euchlanis dilatata Ehrenberg, 1830	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Filinia longiseta (Ehrenberg, 1834)	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	3	2	0	0	1	
Filinia opolensis (Zacharias, 1898)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Filinia terminalis (Plate, 1886)	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	
Gastropus stylifer Imhof, 1891	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Hexarthra bulgarica (Wiszniewski, 1933)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Hexarthra mira (Hudson, 1871)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9	7	1	0	0	0	
Hexarthra sp. Schmarda, 1854	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Kellicottia longispina (Kellicott, 1879)	6	5	4	0	2	3	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Keratella cochlearis (Gosse, 1851)	0	0	0	8	12	12	1	1	0	0	0	1	12	12	1	26	6	4	1	0	0	49	
Keratella quadrata (Müller, 1786)	0	0	1	0	0	1	7	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Keratella tecta (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	3	
Keratella tropica (Apstein, 1907)	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	7	8	0	0	
Lecane bulla (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Lecane closterocerca (Schmarda, 1859)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Lecane luna (Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Lecane quadridentata (Ehrenberg, 1830)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Lepadella ehrenbergii (Perty, 1850)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Lepadella patella (Müller, 1773)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Lepadella sp. Bory de St. Vincent, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Monommata actices Myers, 1930	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Ploesoma hudsoni (Imhof, 1891)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Ploesoma truncatum (Levander, 1894)	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Polyarthra dolichoptera Idelson, 1925	0	0	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	
Polyarthra luminosa Kutikova, 1962	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Polyarthra sp. Ehrenberg, 1834	0	0	0	0	1	4	0	0	0	10	7	2	34	4	26	35	43	18	0	0	4	1	
Polyarthra vulgaris Carlin, 1943	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	1	3	0	0	
Pompholyx sulcata Hudson, 1885	0	0	0	0	0	0	0	0	0	0	0	0	1	23	7	1	1	1	0	2	0	1	

Appendix 2 Continued

Codes	Tri_Jl_16	Tri_Au_16	Tri_Se_16	Veg_Jl_87	Veg_Au_87	Veg_Se_87	Veg_Jn_17	Veg_Jl_84	Veg_Au_17	Vol_Jl_84	Vol_Se_84	Vol_Jl_85	Vol_Au_85	Vol_Se_85	Vol_In_86	Vol_Jl_86	Vol_Au_86	Vou_Jl_16	Vou_Au_16	Vou_Se_16	Yi_Jl_90	Yi_Au_90	Yi_Se_90	
Proalides subtilis Rodewald, 1940	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Scaridium longicaudum (Müller, 1786)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Squatinnella lamellaris (Müller, 1786)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synchaeta sp. Ehrenberg, 1832	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Synchaeta stylata Wierzejski, 1893	29	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichocerca capucina (Wierzejski & Zacharias, 1893)	0	0	0	0	0	0	0	0	0	2	0	1	2	3	5	2	2	0	0	0	0	0	0	1
Trichocerca cylindrica (Imhof, 1891)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichocerca dixonnuttalli (Jennings, 1903)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichocerca pusilla (Jennings, 1903)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0
Trichocerca ruttneri Donner, 1953	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichocerca similis (Wierzejski, 1893)	0	1	2	1	1	3	2	20	11	0	0	1	6	3	9	0	0	0	0	0	0	0	0	0
Trichocerca sp. Lamarck, 1801 ²	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Unidentified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladocera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bosmina (Bosmina) longirostris (O. F. Müller, 1776)	0	0	0	1	1	0	0	0	1	2	1	0	0	0	1	7	0	8	43	28	7	1	2	
Ceriodaphnia dubia Richard, 1894	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceriodaphnia pulchella Sars, 1862	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ceriodaphnia quadrangula (O. F. Müller, 1785)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	12	7
Chydorus sphaericus (O. F. Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coronatella rectangula (Sars, 1862)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Daphnia (Daphnia) cucullata Sars, 1862	0	0	0	2	5	4	1	1	2	2	1	0	2	1	0	1	0	0	0	0	12	36	0	0
Daphnia (Daphnia) galeata Sars, 1864	0	0	0	0	0	0	3	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Daphnia spp. O. F. Mueller, 1785	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diaphanosoma spp. Fischer, 1850	15	10	1	15	20	15	18	8	29	10	15	19	3	6	9	1	3	2	4	1	5	1	1	3
Macrothrix hirsuticornis Norman & Brady, 1867	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Moina micrura Kurz, 1875	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptodora kidtnii (Focke, 1844)	0	0	0	1	0	0	1	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nauplii	16	22	26	5	10	5	12	8	22	10	14	18	6	17	15	12	22	51	15	13	5	10	43	33
Copepodite Calanoida	16	13	13	41	38	25	8	10	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepodite Cyclopoida	0	0	2	0	0	0	2	1	7	27	38	47	7	39	25	9	10	13	0	7	3	13	0	2
Acanthocyclops robustus group (Sars G.O., 1863)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
Arctodiaptomus steindachneri (Richard, 1897)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyclops vicinus vicinus Ulianin, 1875	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucyclops serrulatus serrulatus (Fischer, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0
Eudiaptomus drieschi (Poppe & Mrázek, 1895)	8	9	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eudiaptomus gracilis (Sars G.O., 1863)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Macrocyclops albidus albidus (Jurine, 1820)	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mesocyclops leuckarti leuckarti (Claus, 1857)	0	0	0	0	0	0	0	0	0	2	2	2	2	3	1	1	0	0	0	0	0	0	0	0
Neolovenula alluaudi (Guerne & Richard, 1890)	0	0	0	23	8	24	2	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thermocyclops spp. Kiefer, 1927	0	0	0	0	0	0	1	1	3	3	5	6	1	2	1	1	1	3	1	0	0	0	4	7
Cyclopoida unidentified	0	0	0	3	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 3 Taxa's contribution (%) to total biomass of zooplankton communities from the 17 Greek lakes. Yellow colour indicates the dominating (> 20%) taxa. Codes according to samplings abbreviation in Figure 3.1

Codes	Amu_Jl_16	Amv_Au_16	Amv_Se_16	Doi_Jl_04	Doi_Au_04	Doi_Se_04	Kas_Jl_99	Kas_Au_99	Kas_Se_99	Kas_Jl_16	Kas_Au_16	Kas_Se_16	Kre_Jl_16	Kre_Au_16	Kre_Se_16	Lys_Jl_16	Lys_Au_16	Lys_Se_16	Mgp_Jn_16	Mgp_Jl_16	Mgp_Au_16	Mkp_Jn_90	Mkp_Jl_90	Mkp_Au_90	
Bivalvia	7	6	6	0	0	0	0	0	0	0	0	0	13	50	38	0	0	0	0	0	0	0	0	0	0
Rotifera																									
<i>Anuraeopsis fissa</i> (Gosse, 1851)	0	0	0	0	0	0	0	3	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha ecaudis</i> Perty, 1850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha ovalis</i> (Bergendal, 1892)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha saltans</i> Bartsch, 1870	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna girodi</i> Guerne, 1888	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna priodonta</i> Gosse, 1850	1	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2
<i>Asplanchna</i> spp. Gosse, 1850	0	0	0	8	0	8	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0
<i>Bdelloidea</i> Hudson, 1884	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus angularis</i> Gosse, 1851	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
<i>Brachionus budapestinensis</i> Daday, 1885	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus calyciflorus</i> Pallas, 1766	0	0	0	0	0	1	8	4	0	0	0	1	0	0	0	2	6	0	0	0	0	0	0	0	0
<i>Brachionus cf. quadridentatus</i> Hermann, 1783	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus dimidiatus</i> Bryce, 1931	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus diversicornis</i> (Daday, 1883)	0	0	0	15	1	12	2	7	3	0	22	9	0	0	0	2	2	0	0	0	0	0	0	0	1
<i>Brachionus forficula</i> Wierzejski, 1891	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus ibericus</i> Ciros-Pérez, Gómez & Serra, 2001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus sessilis</i> Varga, 1951	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus urceolaris</i> Müller, 1773	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cephalodella gibba</i> (Ehrenberg, 1830)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cephalodella</i> sp. Bory de St. Vincent, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Collotheca</i> sp. Harring, 1913	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conochilus</i> sp. Ehrenberg, 1834	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conochilus dossuarius</i> Hudson, 1885	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conochilus unicornis</i> Rousselet, 1892	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epiphantes macroura</i> (Barrois & Daday, 1894)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euchlanis dilatata</i> Ehrenberg, 1830	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Filinia longisetata</i> (Ehrenberg, 1834)	0	0	0	0	0	0	0	2	1	0	0	10	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Filinia opoliensis</i> (Zacharias, 1898)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1	2	0	0	0	0	0	0	0
<i>Filinia terminalis</i> (Plate, 1886)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gastropus stylifer</i> Imhof, 1891	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexarthra bulgarica</i> (Wiszniewski, 1933)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexarthra mira</i> (Hudson, 1871)	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexarthra</i> sp. Schmarda, 1854	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Kellicottia longispina</i> (Kellicott, 1879)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Keratella cochlearis</i> (Gosse, 1851)	0	0	0	0	0	0	0	4	6	1	0	4	0	0	0	0	1	1	2	0	0	0	0	0	0
<i>Keratella quadrata</i> (Müller, 1786)	0	0	0	1	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Keratella tecta</i> (Gosse, 1851)	0	0	0	2	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Keratella tropica</i> (Apstein, 1907)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>Lecane bulla</i> (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane closterocerca</i> (Schmarda, 1859)	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane luna</i> (Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Codes	Amv_Jl_16	Amv_Au_16	Amv_Se_16	Doi_Jl_04	Doi_Au_04	Doi_Se_04	Kas_Jl_99	Kas_Au_99	Kas_Se_99	Kas_Jl_16	Kas_Au_16	Kas_Se_16	Kre_Jl_16	Kre_Au_16	Kre_Se_16	Lys_Jl_16	Lys_Au_16	Lys_Se_16	MgP_Jn_16	MgP_Au_16	MgP_Jl_16	Mkp_Jn_90	Mkp_Jl_90	Mkp_Au_90	
<i>Lecane quadridentata</i> (Ehrenberg, 1830)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella ehrenbergii</i> (Perty, 1850)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella patella</i> (Müller, 1773)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella</i> sp. Bory de St. Vincent, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Monommata actices</i> Myers, 1930	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ploesoma hudsoni</i> (Imhof, 1891)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
<i>Ploesoma truncatum</i> (Levander, 1894)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
<i>Polyarthra dolichoptera</i> Idelson, 1925	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	11	10	0	0	0	0	0	0	0
<i>Polyarthra luminosa</i> Kutikova, 1962	0	0	0	0	0	0	0	0	0	0	0	2	3	14	0	0	0	0	0	0	0	0	0	0	0
<i>Polyarthra</i> sp. Ehrenberg, 1834	0	0	0	14	13	17	5	6	25	7	19	17	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Polyarthra vulgaris</i> Carlin, 1943	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pompholyx sulcata</i> Hudson, 1885	0	0	0	3	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Proalides subtilis</i> Rodewald, 1940	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Scardium longicaudum</i> (Müller, 1786)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Squatinnella lamellaris</i> (Müller, 1786)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Synchaeta</i> sp. Ehrenberg, 1832	0	0	0	0	0	0	0	0	0	0	0	6	1	1	0	0	1	0	0	0	0	0	0	0	0
<i>Synchaeta stylata</i> Wierzejski, 1893	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca capucina</i> (Wierzejski & Zacharias, 1893)	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca cylindrica</i> (Imhof, 1891)	0	0	0	0	0	0	0	0	1	1	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca dixonntalli</i> (Jennings, 1903)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca pusilla</i> (Jennings, 1903)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca ruttneri</i> Donner, 1953	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca similis</i> (Wierzejski, 1893)	0	0	0	2	0	0	2	1	0	0	4	2	0	0	0	1	2	0	0	0	0	0	0	0	0
<i>Trichocerca</i> sp. Lamarck, 1801 ³	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unidentified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladocera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bosmina</i> (<i>Bosmina</i>) <i>longirostris</i> (O. F. Müller, 1776)	0	1	0	0	0	0	0	5	2	0	0	1	6	4	4	19	7	5	0	0	0	0	0	0	0
<i>Ceriodaphnia dubia</i> Richard, 1894	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceriodaphnia pulchella</i> Sars, 1862	1	1	5	0	0	0	6	1	2	2	1	1	0	0	0	1	1	1	0	0	0	4	4	4	4
<i>Ceriodaphnia quadrangula</i> (O. F. Müller, 1785)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Chydorus sphaericus</i> (O. F. Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Coronatella rectangula</i> (Sars, 1862)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Daphnia</i> (<i>Daphnia</i>) <i>cucullata</i> Sars, 1862	3	2	2	1	6	0	0	0	0	0	1	1	0	0	0	0	1	1	0	44	21	5	5	5	5
<i>Daphnia</i> (<i>Daphnia</i>) <i>galeata</i> Sars, 1864	0	0	0	0	0	0	0	0	69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Daphnia</i> spp. O. F. Mueller, 1785	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diaphanosoma</i> spp. Fischer, 1850	1	10	5	14	19	6	24	1	4	0	0	0	20	13	11	27	3	7	7	4	21	2	9	10	10
<i>Macrothrix hirsuticornis</i> Norman & Brady, 1867	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Moina micrura</i> Kurz, 1875	0	0	0	0	0	0	0	0	0	0	0	0	0	4	5	0	0	0	0	0	0	0	0	0	0
<i>Leptodora kidtnii</i> (Focke, 1844)	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Copepoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nauplii	2	4	8	25	36	21	22	32	22	1	23	17	28	19	29	20	25	57	10	5	6	5	11	14	14
Copepodite Calanoida	30	13	15	0	2	2	0	0	0	0	0	0	0	0	9	1	1	16	8	13	7	3	10		
Copepodite Cyclopoida	1	1	1	3	18	14	18	31	13	8	7	7	2	3	2	1	7	0	1	2	2	14	20		
<i>Acanthocyclops robustus</i> group (Sars G.O., 1863)	0	0	0	3	2	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Arctodiaptomus steindachneri</i> (Richard, 1897)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	47	65	42	28	20	9		
<i>Cyclops vicinus vicinus</i> Ulyanin, 1875	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eucyclops serrulatus serrulatus</i> (Fischer, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 3 Continued

Codes	Amv_Jl_16	Amv_Au_16	Amv_Se_16	Doi_Jl_04	Doi_Au_04	Doi_Se_04	Kas_Jl_99	Kas_Au_99	Kas_Se_99	Kas_Jl_16	Kas_Au_16	Kas_Se_16	Kre_Jl_16	Kre_Au_16	Kre_Se_16	Lys_Jl_16	Lys_Au_16	Lys_Se_16	MgP_Jn_16	MgP_Au_16	MgP_Se_16	Mkp_Jn_90	Mkp_Au_90	Mkp_Se_90	Mkp_Jl_91	Mkp_Au_91	Mkp_Se_91	
<i>Eudiaptomus drieschi</i> (Poppe & Mrázek, 1895)	37	54	47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eudiaptomus gracilis</i> (Sars G.O., 1863)	0	0	0	3	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Macrocyclops albidus albidus</i> (Jurine, 1820)	17	8	12	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0
<i>Mesocyclops leuckarti leuckarti</i> (Claus, 1857)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16	15
<i>Neolovenula alluaudi</i> (Guerne & Richard, 1890)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12	11
<i>Thermocyclops</i> spp. Kiefer, 1927	0	0	0	2	4	5	4	2	20	9	22	17	10	7	0	0	6	31	8	0	0	0	0	0	0	0	0	
Cyclopoida unidentified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Codes	Mkp_Jl_91	Mkp_Au_91	Mkp_Se_91	Mkp_Jl_92	Mkp_Au_92	Mkp_Se_92	Mkp_Jn_16	Mkp_Au_16	Mkp_Se_16	Oze_Jl_16	Oze_Au_16	Oze_Se_16	Pam_Jl_16	Pam_Au_16	Pam_Se_16	Par_Jl_17	Par_Au_17	Par_Se_17	Pet_Jl_10	Pet_Se_10	Pet_Au_10	Pet_Se_10	Tau_Jl_87	Tau_Au_87	Tau_Se_87			
Bivalvia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Rotifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
<i>Anuraeopsis fissa</i> (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha ecaudis</i> Perty, 1850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha ovalis</i> (Bergendal, 1892)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha saltans</i> Bartsch, 1870	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna girodi</i> Guerne, 1888	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna priodonta</i> Gosse, 1850	0	1	2	1	3	0	0	0	0	0	0	0	0	19	8	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna</i> spp. Gosse, 1850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bdelloidea</i> Hudson, 1884	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus angularis</i> Gosse, 1851	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus budapestinensis</i> Daday, 1885	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus calyciflorus</i> Pallas, 1766	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	9	0
<i>Brachionus cf. quadridentatus</i> Hermann, 1783	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus dimidiatus</i> Bryce, 1931	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus diversicornis</i> (Daday, 1883)	0	0	1	0	0	0	1	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus forficula</i> Wierzejski, 1891	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	
<i>Brachionus ibericus</i> Círos-Pérez, Gómez & Serra, 2001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus sessilis</i> Varga, 1951	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus urceolaris</i> Müller, 1773	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cephalodella gibba</i> (Ehrenberg, 1830)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cephalodella</i> sp. Bory de St. Vincent, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Colloteca</i> sp. Harring, 1913	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conochilus</i> sp. Ehrenberg, 1834	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conochilus dassarius</i> Hudson, 1885	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conochilus unicornis</i> Rousselet, 1892	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epiphantes macroura</i> (Barrois & Daday, 1894)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Euchlanis dilatata</i> Ehrenberg, 1830	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Filinia longisetata</i> (Ehrenberg, 1834)	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	6	0	0	0	0
<i>Filinia opoliensis</i> (Zacharias, 1898)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Filinia terminalis</i> (Plate, 1886)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gastropus stylifer</i> Imhof, 1891	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexarthra bulgarica</i> (Wiszniewski, 1933)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexarthra mira</i> (Hudson, 1871)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexarthra</i> sp. Schmarda, 1854	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Codes	MkP_Jl_91	MkP_Au_91	MkP_Se_91	MkP_Jl_92	MkP_Au_92	MkP_Se_92	MkP_Jn_16	MkP_Jl_16	MkP_Au_16	Oze_Jl_16	Oze_Au_16	Oze_Se_16	Pam_Au_16	Pam_Se_16	Par_Jl_17	Par_Au_17	Par_Se_17	Pet_Jl_10	Pet_Au_10	Pet_Se_10	Tau_Jl_87	Tau_Au_87	Tau_Se_87	
<i>Kellicottia longispina</i> (Kellicott, 1879)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	3
<i>Keratella cochlearis</i> (Gosse, 1851)	0	0	0	0	0	0	3	1	3	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>Keratella quadrata</i> (Müller, 1786)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Keratella tecta</i> (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
<i>Keratella tropica</i> (Apstein, 1907)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane bulla</i> (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane closterocerca</i> (Schmarda, 1859)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane luna</i> (Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane quadridentata</i> (Ehrenberg, 1830)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella ehrenbergii</i> (Perty, 1850)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella patella</i> (Müller, 1773)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella</i> sp. Bory de St. Vincent, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Monommata actices</i> Myers, 1930	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ploesoma hudsoni</i> (Imhof, 1891)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ploesoma truncatum</i> (Levander, 1894)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polyarthra dolichoptera</i> Idelson, 1925	0	0	0	0	0	0	0	0	0	4	4	1	1	0	0	0	0	0	0	0	0	0	0	0
<i>Polyarthra luminosa</i> Kutikova, 1962	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polyarthra</i> sp. Ehrenberg, 1834	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	39	0	2	7	21	0	0	0	7
<i>Polyarthra vulgaris</i> Carlin, 1943	0	0	0	0	0	0	1	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Pompholyx sulcata</i> Hudson, 1885	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Proalides subtilis</i> Rodewald, 1940	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Scardium longicaudum</i> (Müller, 1786)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Squatinnella lamellaris</i> (Müller, 1786)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Synchaeta</i> sp. Ehrenberg, 1832	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	63	0	0	0	0	0
<i>Synchaeta stylata</i> Wierzejski, 1893	0	0	0	0	0	0	0	0	0	4	3	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca capucina</i> (Wierzejski & Zacharias, 1893)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca cylindrica</i> (Imhof, 1891)	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0
<i>Trichocerca dixonntalli</i> (Jennings, 1903)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca pusilla</i> (Jennings, 1903)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca ruttneri</i> Donner, 1953	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca similis</i> (Wierzejski, 1893)	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca</i> sp. Lamarck, 1801 ²	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unidentified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladocera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bosmina</i> (<i>Bosmina</i>) <i>longirostris</i> (O. F. Müller, 1776)	0	0	0	0	0	0	0	1	1	0	0	0	0	2	1	3	1	12	17	16	0	0	0	0
<i>Ceriodaphnia dubia</i> Richard, 1894	0	0	0	0	0	0	0	2	7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceriodaphnia pulchella</i> Sars, 1862	0	12	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
<i>Ceriodaphnia quadrangula</i> (O. F. Müller, 1785)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11	0	0	0	0	0
<i>Chydorus sphaericus</i> (O. F. Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0	0	2	30	3	0	0	0	0	0	0	0	0
<i>Coronatella rectangula</i> (Sars, 1862)	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Daphnia</i> (<i>Daphnia</i>) <i>cucullata</i> Sars, 1862	7	10	8	12	8	3	0	0	0	0	0	0	0	25	20	47	0	0	0	0	0	0	0	0
<i>Daphnia</i> (<i>Daphnia</i>) <i>galeata</i> Sars, 1864	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Daphnia</i> spp. O. F. Mueller, 1785	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	55	56	46
<i>Diaphanosoma</i> spp. Fischer, 1850	0	0	14	11	29	27	0	0	8	12	1	3	13	3	16	21	0	1	19	10	0	19	14	3
<i>Macrothrix hirsuticornis</i> Norman & Brady, 1867	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Moina micrura</i> Kurz, 1875	0	0	0	0	0	0	0	0	0	1	0	0	2	0	1	1	1	0	0	0	0	0	0	0
<i>Leptodora kidnii</i> (Focke, 1844)	0	0	0	0	2	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 3 Continued

Codes	Tri_Jl_16	Tri_Au_16	Tri_Se_16	Veg_Jl_87	Veg_Se_87	Veg_In_17	Veg_Jl_17	Veg_Au_17	Vol_Jl_84	Vol_Se_84	Vol_In_16	Vol_Au_16	Vol_Se_16	Vol_Jl_17	Vol_Au_17	Vol_Se_17	Vou_Jl_16	Vou_Au_16	Vou_Se_16	Yii_Jl_90	Yii_Au_90	Yii_Se_90	
Copepoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nauplii	15	18	19	7	13	18	19	25	8	22	2	4	11	24	4	2	3	1	8	0	0	0	0
Copepodite Calanoida	8	16	11	15	9	19	15	6	11	23	0	0	0	0	3	7	7	17	6	16	6	39	19
Copepodite Cyclopoida	8	13	12	6	2	4	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Acanthocyclops robustus</i> group (Sars G.O., 1863)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Arctodiaptomus steindachneri</i> (Richard, 1897)	52	20	15	40	24	14	29	56	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclops vicinus vicinus</i> Uljanin, 1875	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	16
<i>Eucyclops serrulatus serrulatus</i> (Fischer, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eudiaptomus drieschi</i> (Poppe & Mrázek, 1895)	0	0	0	0	0	0	0	0	0	39	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eudiaptomus gracilis</i> (Sars G.O., 1863)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Macrocyclops albidus albidus</i> (Jurine, 1820)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mesocyclops leuckarti leuckarti</i> (Claus, 1857)	3	10	15	8	8	11	26	6	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Neolovenula alluaudi</i> (Guerne & Richard, 1890)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Thermocyclops</i> spp. Kiefer, 1927	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19	8	4	0	0	0	0	0
Cyclopoida unidentified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3
Bivalvia	3	16	12	0	0	0	12	13	2	1	1	0	0	0	5	10	9	1	0	0	0	0	0
Rotifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Anuraeopsis fissa</i> (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha ecaudis</i> Perty, 1850	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha ovalis</i> (Bergendal, 1892)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ascomorpha saltans</i> Bartsch, 1870	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna girodi</i> Guerne, 1888	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Asplanchna priodonta</i> Gosse, 1850	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	7	9
<i>Asplanchna</i> spp. Gosse, 1850	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bdelloidea</i> Hudson, 1884	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus angularis</i> Gosse, 1851	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus budapestinensis</i> Daday, 1885	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
<i>Brachionus calyciflorus</i> Pallas, 1766	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus cf. quadridentatus</i> Hermann, 1783	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus dimidiatus</i> Bryce, 1931	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus diversicornis</i> (Daday, 1883)	0	0	0	0	0	0	0	0	0	23	6	2	4	1	0	0	1	0	0	1	3	0	0
<i>Brachionus farficalis</i> Wierzejski, 1891	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus ibericus</i> Círos-Pérez, Gómez & Serra, 2001	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus sessilis</i> Varga, 1951	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Brachionus urceolaris</i> Müller, 1773	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cephalodella gibba</i> (Ehrenberg, 1830)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cephalodella</i> sp. Bory de St. Vincent, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Colloteca</i> sp. Harring, 1913	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
<i>Conochilus</i> sp. Ehrenberg, 1834	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conochilus dassarius</i> Hudson, 1885	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Conochilus unicornis</i> Rousselet, 1892	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Epiphantes macroura</i> (Barrois & Daday, 1894)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 3 Continued

Codes	Tri_Jl_16	Tri_Au_16	Tri_Se_16	Veg_Jl_87	Veg_Au_87	Veg_Se_87	Veg_Jn_17	Veg_Jl_17	Veg_Au_17	Veg_Se_84	Vol_Jl_84	Vol_Au_84	Vol_Se_84	Vol_Jl_85	Vol_Au_85	Vol_Se_85	Vol_Jl_86	Vol_Au_86	Vol_Se_86	Vol_Jl_16	Vol_Au_16	Vol_Se_16	Vol_Jl_90	Vol_Au_90	Vol_Se_90
<i>Euchlanis dilatata</i> Ehrenberg, 1830	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Filinia longisetata</i> (Ehrenberg, 1834)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
<i>Filinia opoliensis</i> (Zacharias, 1898)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Filinia terminalis</i> (Plate, 1886)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Gastropus stylifer</i> Imhof, 1891	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexarthra bulgarica</i> (Wiszniewski, 1933)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Hexarthra mira</i> (Hudson, 1871)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0
<i>Hexarthra</i> sp. Schmarda, 1854	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Kellicottia longispina</i> (Kellicott, 1879)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Keratella cochlearis</i> (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
<i>Keratella quadrata</i> (Müller, 1786)	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Keratella tecta</i> (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Keratella tropica</i> (Apstein, 1907)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Lecane bulla</i> (Gosse, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane closterocerca</i> (Schmarda, 1859)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane luna</i> (Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lecane quadridentata</i> (Ehrenberg, 1830)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella ehrenbergii</i> (Perty, 1850)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella patella</i> (Müller, 1773)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Lepadella</i> sp. Bory de St. Vincent, 1826	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Monommata actices</i> Myers, 1930	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ploesoma hudsoni</i> (Imhof, 1891)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ploesoma truncatum</i> (Levander, 1894)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polyartha dolichoptera</i> Idelson, 1925	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polyartha luminosa</i> Kutikova, 1962	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Polyartha</i> sp. Ehrenberg, 1834	0	0	0	0	0	0	0	0	0	0	3	1	0	19	1	8	21	20	8	0	0	0	1	0	1
<i>Polyartha vulgaris</i> Carlin, 1943	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0
<i>Pompholyx sulcata</i> Hudson, 1885	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
<i>Proalides subtilis</i> Rodewald, 1940	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Scardium longicaudum</i> (Müller, 1786)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Squatinella lamellaris</i> (Müller, 1786)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Synchaeta</i> sp. Ehrenberg, 1832	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Synchaeta stylata</i> Wierzejski, 1893	11	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca capucina</i> (Wierzejski & Zacharias, 1893)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	0
<i>Trichocerca cylindrica</i> (Imhof, 1891)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca dixonnuttalli</i> (Jennings, 1903)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca pusilla</i> (Jennings, 1903)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca ruttneri</i> Donner, 1953	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Trichocerca similis</i> (Wierzejski, 1893)	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0
<i>Trichocerca</i> sp. Lamarck, 1801 ³	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unidentified	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cladocera</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bosmina</i> (<i>Bosmina</i>) <i>longirostris</i> (O. F. Müller, 1776)	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	3	9	0	10	35	24	8	2	4	
<i>Ceriodaphnia dubia</i> Richard, 1894	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceriodaphnia pulchella</i> Sars, 1862	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Ceriodaphnia quadrangula</i> (O. F. Müller, 1785)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	26	13	
<i>Chydorus sphaericus</i> (O. F. Müller, 1776)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	27	0	0	0	

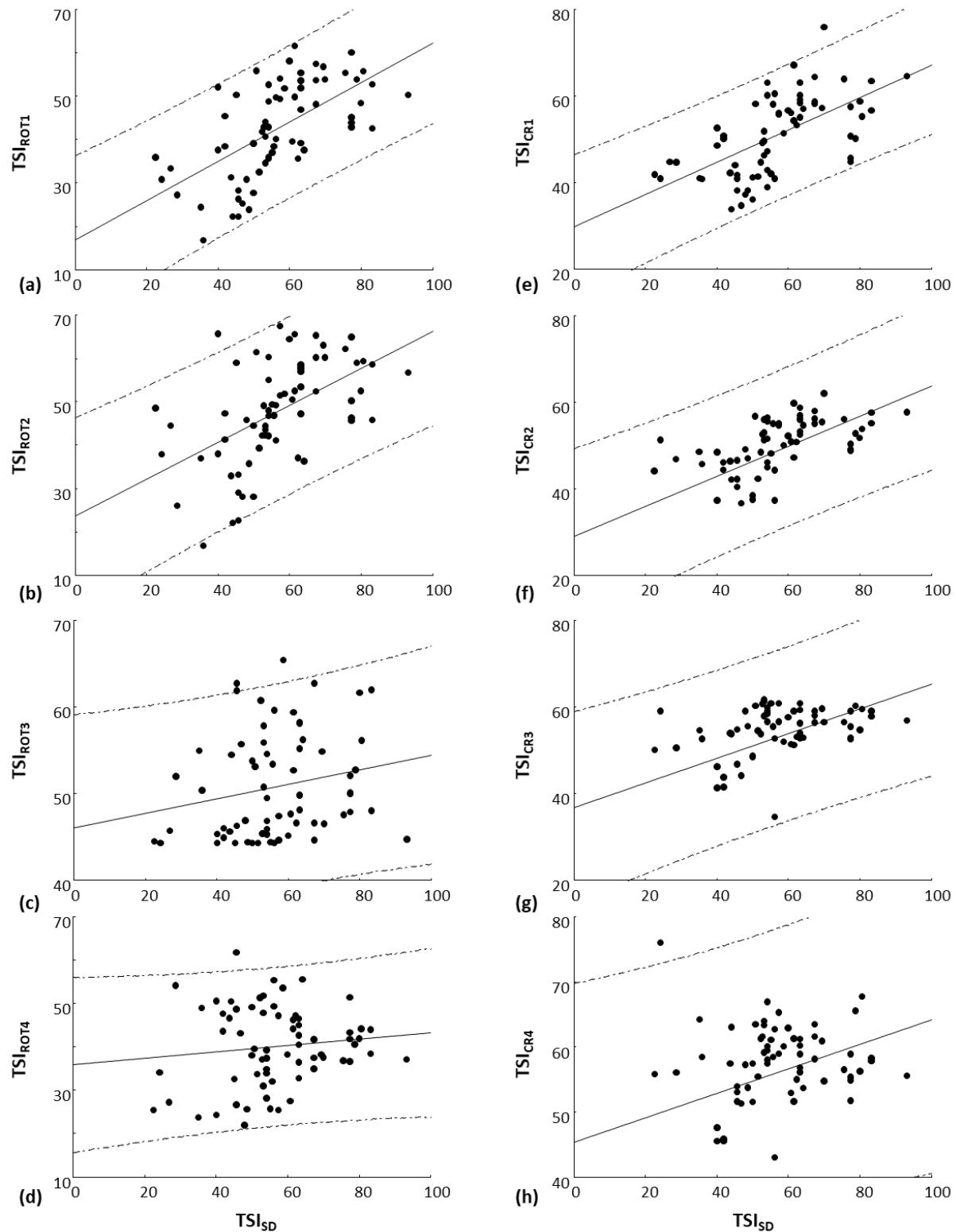
Codes	Tri_Jl_16	Tri_Au_16	Tri_Se_16	Veg_Jl_87	Veg_Au_87	Veg_Se_87	Veg_Jn_17	Veg_Jl_17	Veg_Au_84	Veg_Se_84	Vol_Jl_84	Vol_Au_84	Vol_Se_84	Vol_Jl_85	Vol_Au_85	Vol_Se_85	Vol_Jn_86	Vol_Jl_86	Vol_Au_86	Vol_Se_86	Vol_Au_16	Vol_Se_16	Vol_Au_90	Vol_Se_90
<i>Coronatella rectangula</i> (Sars, 1862)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0
<i>Daphnia (Daphnia) cucullata</i> Sars, 1862	0	0	0	1	10	4	0	1	2	4	3	0	20	2	0	4	3	0	0	0	0	3	0	2
<i>Daphnia (Daphnia) galeata</i> Sars, 1864	0	0	0	0	0	0	20	0	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Daphnia</i> spp. O. F. Müller, 1785	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Diaphanosoma</i> spp. Fischer, 1850	13	8	1	5	12	5	18	9	23	14	28	18	15	15	19	3	7	5	22	3	13	2	2	13
<i>Macrothrix hirsuticornis</i> Norman & Brady, 1867	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
<i>Moina micrura</i> Kurz, 1875	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0
<i>Leptodora kidtnii</i> (Focke, 1844)	0	0	0	5	0	4	12	22	26	3	3	3	0	0	3	0	0	0	0	0	0	0	0	0
<i>Copepoda</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Nauplii	6	9	7	1	4	1	3	2	4	7	8	10	7	16	13	19	23	50	16	7	3	6	43	25
Copepodite Calanoida	19	16	12	20	34	14	12	24	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepodite Cyclopoida	1	0	1	0	0	0	1	0	2	28	30	58	13	45	30	21	18	21	0	10	6	16	0	4
<i>Acanthocyclops robustus</i> group (Sars G.O., 1863)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	35	31	20	0	0	0
<i>Arctodiaptomus steindachneri</i> (Richard, 1897)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Cyclops vicinus vicinus</i> Ulljanin, 1875	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eucyclops serrulatus serrulatus</i> (Fischer, 1851)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	55	0	0
<i>Eudiaptomus drieschi</i> (Poppe & Mrázek, 1895)	40	47	29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Eudiaptomus gracilis</i> (Sars G.O., 1863)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Macrocyclops albidus albidus</i> (Jurine, 1820)	6	0	37	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Mesocyclops leuckarti leuckarti</i> (Claus, 1857)	0	0	0	0	0	0	0	0	0	7	8	0	15	12	15	9	5	2	0	0	0	1	1	3
<i>Neolovenula alluaudi</i> (Guerne & Richard, 1890)	0	0	0	63	35	69	19	27	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>Thermocyclops</i> spp. Kiefer, 1927	0	0	0	0	0	0	1	1	2	8	8	9	5	6	4	6	6	12	4	1	1	0	18	24
Cyclopoida unidentified	0	0	0	0	4	4	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 4 The values of secchi depth (SD), phytoplankton biomass (PB) (mg/L), the percentage contribution of Cyanobacteria (% CYANO) and trophic state indices (TSI) for each zooplankton - phytoplankton sampling for the 17 studied Greek lakes. Codes according to samplings abbreviation in Figure 3.1, m.v.: missing value.

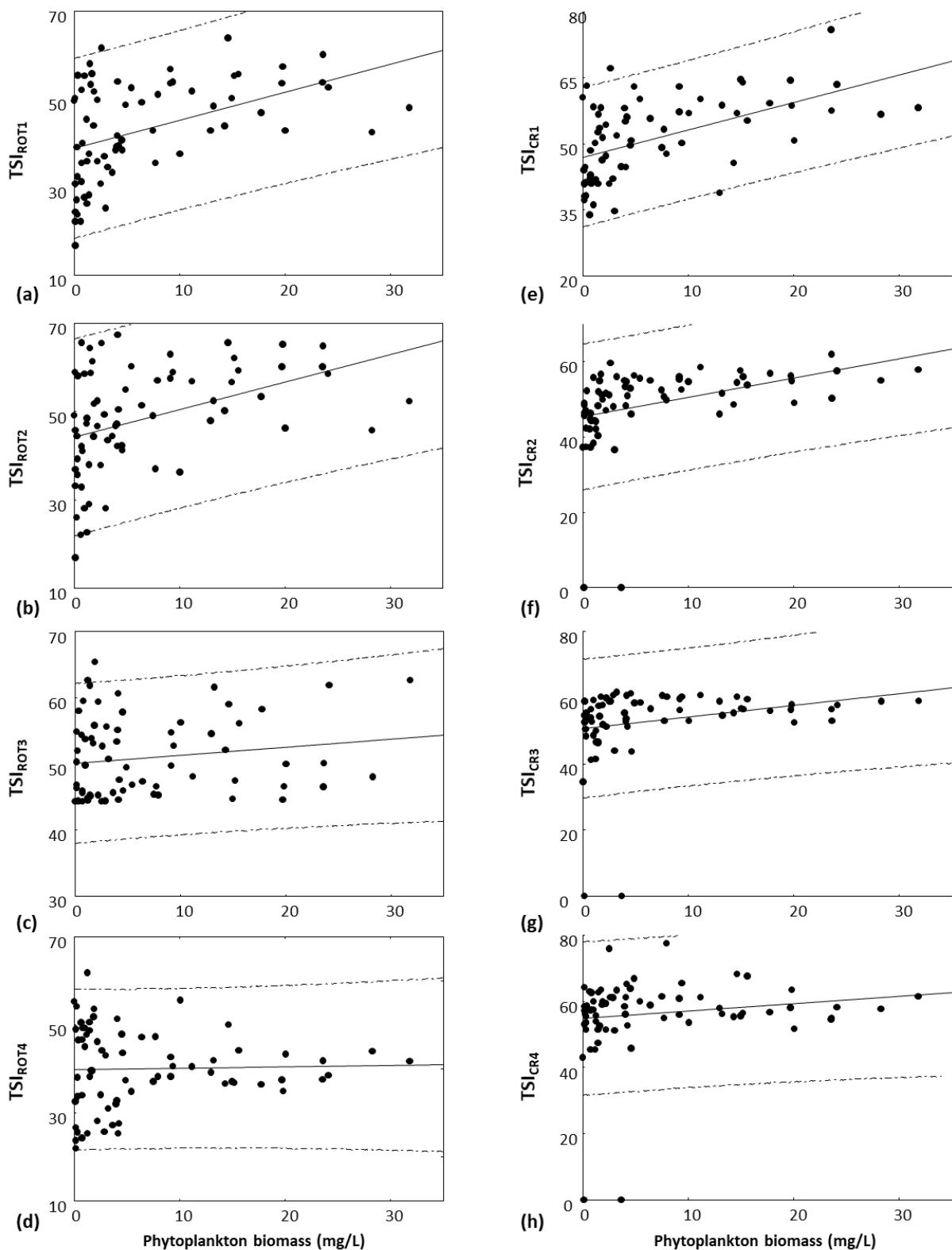
Codes	SD	TSI _{SD}	PB	%CYANO	TSI _{ROT1}	TSI _{ROT2}	TSI _{ROT3}	TSI _{ROT4}	TSI _{ROT}	TSI _{CR1}	TSI _{CR2}	TSI _{CR3}	TSI _{CR4}	TSI _{CR}	TSI _{ZOO}
Amv_Jl_16	5.60	35.15	0.12	36.94	24.42	36.93	54.92	23.77	35.01	41.03	48.58	54.56	64.24	52.10	37.74
Amv_Au_16	8.80	28.62	0.21	75.98	27.22	25.99	51.97	54.09	39.82	44.75	46.87	50.60	56.06	49.57	36.21
Amv_Se_16	5.30	35.94	0.14	75.28	16.82	16.87	50.35	48.97	33.25	40.79	45.68	52.65	58.49	49.40	30.04
Doi_Jl_04	0.30	77.37	23.61	29.65	60.09	64.92	50.10	41.83	54.23	57.62	50.29	52.99	54.91	53.95	58.23
Doi_Au_04	0.60	67.37	19.70	26.40	53.72	60.21	44.60	37.50	49.00	64.40	56.22	56.40	58.11	58.78	58.64
Doi_Se_04	0.60	67.37	19.80	63.13	57.42	65.28	46.60	34.96	51.06	58.61	54.98	58.03	63.51	58.78	59.07
Kas_Jl_99	0.34	75.56	15.20	90.91	55.28	62.13	47.50	36.86	50.45	63.91	56.10	56.52	56.49	58.25	59.36
Kas_Au_99	0.24	80.59	15.60	85.77	55.73	59.42	56.10	44.14	53.85	55.18	53.82	59.49	67.71	59.05	56.04
Kas_Se_99	0.27	78.78	9.38	94.17	53.92	59.03	52.75	40.56	51.56	50.17	52.70	60.25	65.56	57.17	53.95
Kas_Jl_16	m.v.	m.v.	1.57	39.08	53.40	58.86	53.94	39.65	51.46	53.61	54.96	54.44	52.66	53.92	55.21
Kas_Au_16	m.v.	m.v.	7.94	95.73	51.11	57.15	45.23	38.22	47.93	47.82	49.85	60.31	77.62	58.90	51.48
Kas_Se_16	m.v.	m.v.	14.60	80.70	63.84	65.73	59.05	49.94	59.64	57.05	54.53	60.31	68.27	60.04	60.29
Kre_Jl_16	1.50	54.15	0.71	0.00	35.56	42.10	45.93	33.97	39.39	42.99	44.88	56.58	57.42	50.47	41.38
Kre_Au_16	1.80	51.52	0.32	0.00	32.50	39.34	44.30	33.76	37.47	41.34	42.34	54.48	55.49	48.41	38.88
Kre_Se_16	2.00	50.00	0.27	0.00	39.08	44.50	44.30	37.98	41.46	41.16	37.50	48.41	51.56	44.66	40.56
Lys_Jl_16	0.90	61.52	4.89	99.90	49.87	52.47	59.39	46.18	51.98	54.31	47.14	51.23	51.58	51.07	50.95
Lys_Au_16	0.90	61.52	5.81	15.71	61.67	65.65	52.65	44.13	56.03	67.13	59.72	58.92	61.23	61.75	63.54
Lys_Se_16	0.80	63.22	0.81	100.00	55.42	58.17	58.07	46.51	54.54	63.14	52.45	53.92	58.88	57.10	57.30
MgP_Jn_16	1.70	52.34	4.08	64.30	41.83	42.22	60.73	51.29	49.02	44.72	48.51	53.68	61.24	52.04	44.32
MgP_Jl_16	3.00	44.15	0.64	3.50	22.21	21.98	54.42	50.47	37.27	33.89	42.17	53.70	63.03	48.20	30.06
MgP_Au_16	3.10	43.68	0.74	30.94	31.22	32.82	45.66	46.61	39.08	42.18	46.27	53.91	57.48	49.96	38.12
MkP_Jn_90	0.75	64.15	10.04	88.44	37.58	36.36	56.23	55.57	46.43	57.04	54.71	52.92	53.67	54.59	46.42
MkP_Jl_90	0.80	63.22	17.75	79.79	46.97	53.50	58.27	36.46	48.80	59.22	56.93	56.16	56.81	57.28	54.16
MkP_Au_90	0.60	67.37	31.82	92.25	48.12	52.41	62.71	41.71	51.24	58.17	58.02	59.12	61.53	59.21	54.18
MkP_Jl_91	1.10	58.62	1.91	69.74	51.78	51.88	65.40	53.54	55.65	51.44	50.06	51.96	60.07	53.38	51.29
MkP_Au_91	1.35	55.67	3.93	66.35	38.45	46.82	53.37	31.98	42.66	58.11	55.00	55.46	58.47	56.76	49.59
MkP_Se_91	1.20	57.37	6.39	81.79	49.25	51.44	47.42	47.17	48.82	55.75	55.11	56.65	58.97	56.62	52.89
MkP_Jl_92	0.80	63.22	4.03	75.57	39.20	47.26	55.12	32.75	43.58	55.09	53.32	52.83	56.17	54.35	48.72
MkP_Au_92	0.95	60.74	4.21	64.42	39.49	50.43	47.61	27.52	41.26	56.03	50.91	51.31	52.86	52.78	49.22
MkP_Se_92	0.85	62.34	7.71	86.78	35.54	37.08	46.59	47.30	41.63	53.31	50.87	53.11	54.98	53.07	44.20
MkP_Jn_16	1.30	56.21	0.80	63.76	40.02	41.02	59.59	49.32	47.49	40.94	44.28	52.78	62.72	50.18	41.56
MkP_Jl_16	2.00	50.00	0.97	42.81	27.68	28.18	53.76	49.14	39.69	36.11	38.50	48.65	57.48	45.18	32.62
MkP_Au_16	1.60	53.22	1.82	48.13	44.00	44.35	55.87	51.72	48.99	46.28	51.27	57.86	59.12	53.63	46.48
Oze_Jl_16	1.30	56.21	m.v.	m.v.	49.72	49.16	44.30	55.26	49.61	60.56	37.27	34.60	43.04	43.87	49.18
Oze_Au_16	2.80	45.15	0.072	24.20	50.22	59.03	44.30	32.59	46.54	44.01	0.00	0.00	11.00	38.31	

Appendix 4 continued

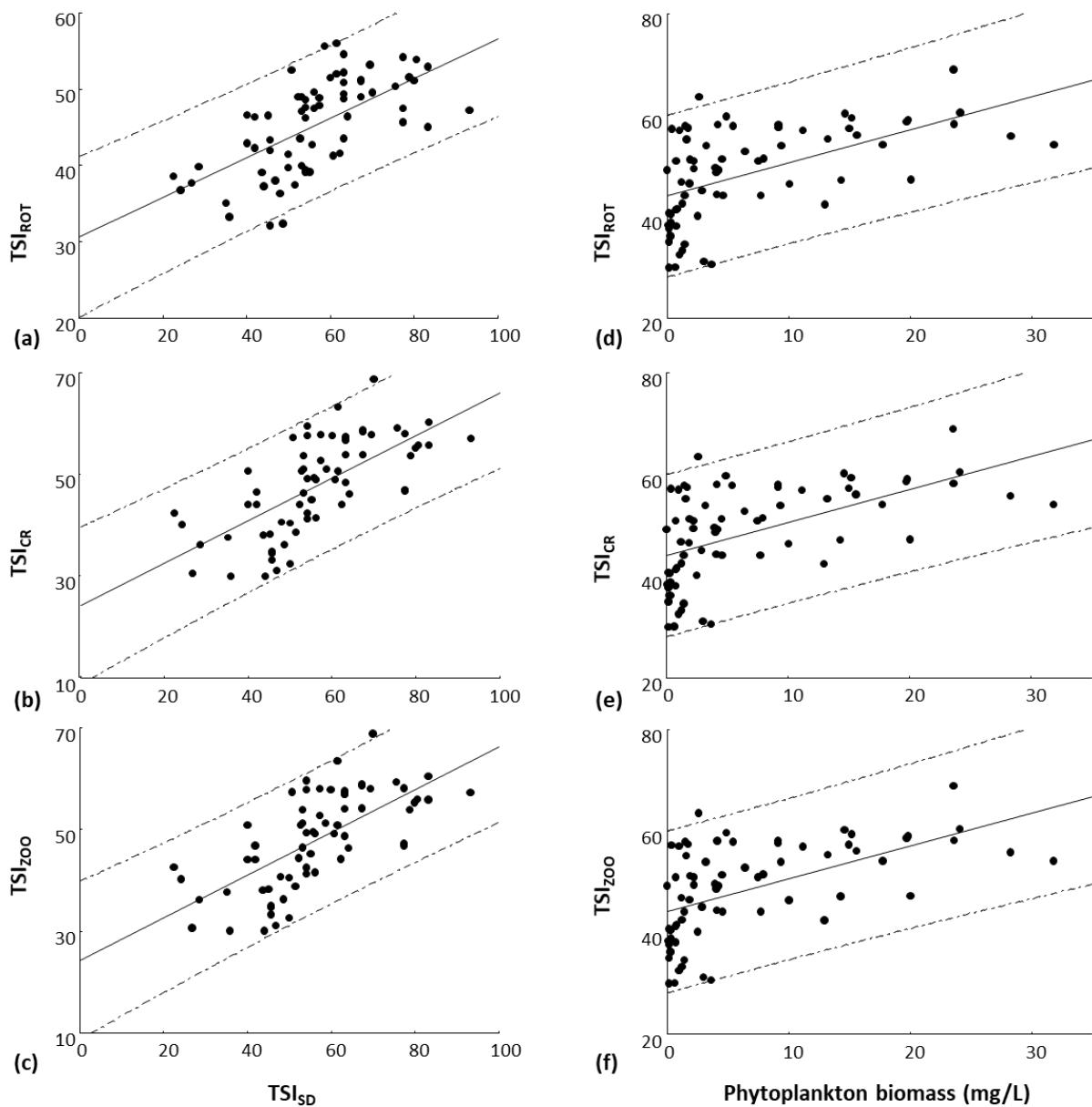
Codes	SD	TSI _{SD}	PB	%CYANO	TSI _{ROT1}	TSI _{ROT2}	TSI _{ROT3}	TSI _{ROT4}	TSI _{ROT}	TSI _{CR1}	TSI _{CR2}	TSI _{CR3}	TSI _{CR4}	TSI _{CR}	TSI _{ZOO}
Oze_Se_16	4.00	40.00	0.73	0.00	52.11	65.73	44.30	24.27	46.60	48.56	37.27	41.26	45.50	43.15	50.92
Pam_Jl_16	0.30	77.37	14.31	100.00	43.97	50.28	52.10	36.65	45.75	45.67	48.72	55.39	55.39	51.29	47.16
Pam_Au_16	0.10	93.22	14.96	99.31	50.26	56.79	44.73	37.09	47.22	64.52	57.73	56.84	55.62	58.68	57.32
Pam_Se_16	0.30	77.37	20.04	98.70	42.86	46.32	49.96	43.24	45.59	50.73	49.08	52.58	51.77	51.04	47.25
Par_Jl_17	0.80	63.22	0.97	53.45	55.30	58.62	49.80	45.05	52.19	58.45	55.90	59.28	60.17	58.45	57.07
Par_Au_17	1.00	60.00	1.50	40.37	58.12	64.45	45.16	38.29	51.51	56.76	52.18	57.61	62.87	57.36	57.88
Par_Se_17	1.20	57.37	4.12	54.58	54.00	67.44	44.60	25.35	47.85	56.18	54.66	60.76	65.25	59.21	58.07
Pet_Jl_10	0.80	63.22	9.14	67.82	53.54	57.60	49.77	42.59	50.87	63.10	56.15	56.26	56.02	57.88	57.60
Pet_Au_10	0.80	63.22	11.18	77.31	51.92	56.92	48.09	40.47	49.35	60.12	58.67	60.82	61.21	60.21	56.91
Pet_Se_10	0.52	69.43	9.16	71.90	56.81	63.07	54.80	38.25	53.23	57.26	55.40	59.64	60.91	58.30	58.13
Tav_Jl_87	2.70	45.67	0.14	0.00	22.20	33.25	46.31	26.69	32.11	38.11	46.47	54.81	53.06	48.11	35.00
Tav_Au_87	2.20	48.62	0.30	1.02	23.90	35.80	44.40	25.54	32.41	38.27	47.08	55.53	53.72	48.65	36.26
Tav_Se_87	2.30	47.98	0.12	2.56	30.83	45.74	46.86	21.94	36.34	37.27	49.08	58.92	57.23	50.62	40.73
Tri_Jl_16	13.50	22.45	1.20	68.11	35.91	48.50	44.47	25.35	38.56	41.90	44.08	50.10	55.85	47.98	42.60
Tri_Au_16	10.00	26.78	3.65	88.60	33.44	44.42	45.68	27.22	37.69	44.78	0.00	0.00	0.00	11.20	30.66
Tri_Se_16	12.00	24.15	2.49	99.96	30.79	37.90	44.30	34.10	36.77	40.93	51.30	58.95	75.98	56.79	40.23
Veg_Jl_87	2.70	45.67	1.23	78.15	26.32	22.72	62.70	61.70	43.36	41.78	42.27	46.73	54.00	46.20	33.27
Veg_Au_87	2.70	45.67	1.43	34.64	28.29	28.99	61.87	48.67	41.96	40.91	40.39	46.83	51.58	44.93	34.64
Veg_Se_87	2.50	46.78	3.02	58.54	25.30	28.02	55.65	43.06	38.01	34.77	36.68	44.12	51.31	41.72	31.19
Veg_In_17	3.50	41.93	4.564	5.35	38.41	41.25	45.94	43.59	42.30	50.85	46.06	43.74	45.83	46.62	44.14
Veg_Jl_17	3.50	41.93	1.164	16.46	45.47	47.32	44.89	47.69	46.34	50.12	44.34	41.47	45.50	45.36	46.81
Veg_Au_17	4.00	40.00	1.442	35.79	37.58	38.00	45.32	50.63	42.88	52.67	48.42	46.25	47.59	48.73	44.17
Vol_Jl_84	1.40	55.15	2.89	74.35	37.03	49.38	44.41	25.73	39.14	42.14	48.19	60.80	61.10	53.06	44.18
Vol_Au_84	1.50	54.15	2.19	89.61	35.94	46.67	45.27	28.17	39.01	47.32	51.57	59.97	59.46	54.58	45.37
Vol_Se_84	1.60	53.22	3.19	43.09	34.59	43.57	50.75	30.98	39.97	51.93	56.00	61.65	63.36	58.24	46.52
Vol_Jl_85	1.50	54.15	12.96	87.76	42.85	48.05	54.54	39.20	46.16	38.91	46.09	59.03	58.02	50.51	43.97
Vol_Au_85	1.60	53.22	4.54	62.39	40.70	42.30	57.85	47.77	47.16	49.64	53.00	61.38	63.96	56.99	46.41
Vol_Se_85	1.65	52.78	7.49	25.43	42.84	49.05	45.36	37.03	43.57	49.22	52.55	60.65	61.61	56.01	48.42
Vol_In_86	1.90	50.74	1.71	17.34	55.82	61.48	53.13	39.51	52.48	58.26	56.71	60.23	63.51	59.68	58.07
Vol_Jl_86	1.50	54.15	5.43	63.30	52.59	60.35	46.80	34.79	48.63	60.12	55.60	58.61	60.02	58.59	57.17
Vol_Au_86	1.50	54.15	4.88	56.61	48.72	55.06	49.44	37.33	47.64	63.03	56.42	58.34	66.93	61.18	55.81
Vou_Jl_16	0.30	77.37	74.88	68.87	45.09	45.60	47.89	51.39	47.49	44.78	48.92	59.01	58.87	52.90	46.10
Vou_Au_16	0.20	83.22	28.29	65.77	42.55	45.72	48.02	43.92	45.05	56.73	55.09	58.97	57.82	57.15	50.02
Vou_Se_16	0.50	70.00	23.56	60.90	53.84	60.28	46.51	37.61	49.56	75.91	62.03	56.54	54.75	62.31	63.02
Yli_Jl_90	m.v.	m.v.	m.v.	52.48	48.66	64.38	66.93	58.11	58.45	61.85	62.05	63.95	61.57	55.36	
Yli_Au_90	0.25	80.00	13.23	0.02	48.39	52.54	61.65	41.94	51.13	58.79	51.79	54.71	56.23	55.38	52.88
Yli_Se_90	0.20	83.22	24.10	0.01	52.76	58.61	61.94	38.48	52.95	63.53	57.53	57.81	58.25	59.28	58.11



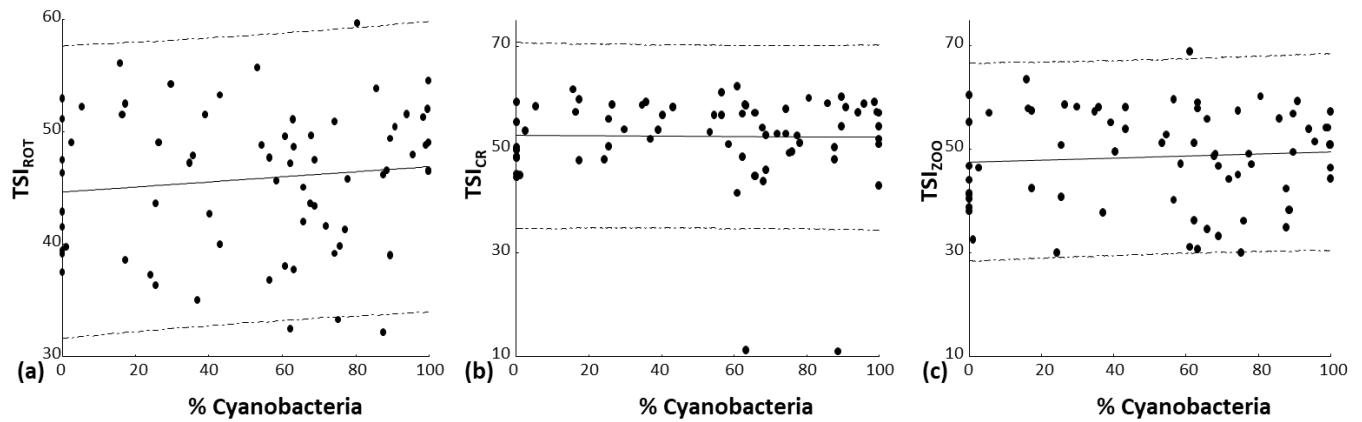
Appendix 5 Scatter plots of TSI_{ROT} formulae [(a) TSI_{ROT1}, (b) TSI_{ROT2}, (c) TSI_{ROT3} and (d) TSI_{ROT4}] and TSI_{CR} formulae [(e) TSI_{CR1}, (f) TSI_{CR2}, (g) TSI_{CR3} and (h) TSI_{CR4}] against TSI_{SD}, the solid line indicates the linear regression line and the dashed lines indicate the (95%) confidence and prediction limits of the model



Appendix 6 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 phytoplankton biomass (mg/L), the solid line indicates the linear regression line and the dashed lines indicate the (95%) confidence and prediction limits of the model



Appendix 7 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 line indicates the linear regression line and the dashed lines indicate the (95%) confidence and prediction limits of the model



Appendix 8 Scatter plots of (a) TSI_{ROT} , (b) TSI_{CR} and (c) TSI_{ZOO} against the percentage contribution of cyanobacteria to total phytoplankton biomass, the solid line indicates the linear regression line and the dashed lines indicate the (95%) confidence and prediction limits of the model

Appendix 9 The trophic categories identified for each sampling using different tropic state indices [TSI_{SD}, mean summer phytoplankton biomass (PB), TSI_{ROT}, TSI_{CR} and TSI_{zoo}]. Codes according to samplings abbreviation in Figure 3.1

Codes	TSI _{SD}	PB	TSI _{ROT}	TSI _{CR}	TSI _{zoo}	Codes	TSI _{SD}	PB	TSI _{ROT}	TSI _{CR}	TSI _{zoo}
Amv_Jl_16	Oligo	Oligo	Meso	Meso-Eu	Meso	Pam_Jl_16	Hyper	Hyper	Meso-Eu	Meso-Eu	Meso-Eu
Amv_Au_16	Oligo	Oligo	Meso	Meso-Eu	Meso	Pam_Au_16	Hyper	Hyper	Meso-Eu	Eu	Eu
Amv_Se_16	Oligo	Oligo	Meso	Meso-Eu	Meso	Pam_Se_16	Hyper	Hyper	Meso-Eu	Meso-Eu	Meso-Eu
Doi_Jl_04	Hyper	Hyper	Meso-Eu	Meso-Eu	Eu	Par_Jl_17	Eu	Meso	Meso-Eu	Eu	Eu
Doi_Au_04	Eu	Hyper	Meso-Eu	Eu	Eu	Par_Au_17	Eu	Meso	Meso-Eu	Eu	Eu
Doi_Se_04	Eu	Hyper	Meso-Eu	Eu	Eu	Par_Se_17	Eu	Meso	Meso-Eu	Eu	Eu
Kas_Jl_99	Hyper	Eu-Hyper	Meso-Eu	Eu	Eu	Pet_Jl_10	Eu	Eu	Meso-Eu	Eu	Eu
Kas_Au_99	Hyper	Eu-Hyper	Meso-Eu	Eu	Eu	Pet_Au_10	Eu	Eu	Meso-Eu	Eu	Eu
Kas_Se_99	Hyper	Eu-Hyper	Meso-Eu	Eu	Meso-Eu	Pet_Se_10	Eu	Eu	Meso-Eu	Eu	Eu
Kas_Jl_16	m.d	Eu	Meso-Eu	Meso-Eu	Eu	Tav_Jl_87	Meso	Oligo	Meso	Meso-Eu	Meso
Kas_Au_16	m.d	Eu	Meso-Eu	Eu	Meso-Eu	Tav_Au_87	Meso	Oligo	Meso	Meso-Eu	Meso
Kas_Se_16	m.d	Eu	Eu	Eu	Eu	Tav_Se_87	Meso	Oligo	Meso	Meso-Eu	Meso
Kre_Jl_16	Eu	Oligo	Meso	Meso-Eu	Meso	Tri_Jl_16	Oligo	Eu	Meso	Meso-Eu	Meso
Kre_Au_16	Eu	Oligo	Meso	Meso-Eu	Meso	Tri_Au_16	Oligo	Eu	Meso	Meso	Meso
Kre_Se_16	Eu	Oligo	Meso	Meso	Meso	Tri_Se_16	Oligo	Eu	Meso	Eu	Meso
Lys_Jl_16	Eu	Meso	Meso	Meso-Eu	Meso-Eu	Veg_Jl_87	Meso	Meso-Eu	Meso	Meso-Eu	Meso
Lys_Au_16	Eu	Meso	Eu	Eu	Eu	Veg_Au_87	Meso	Meso-Eu	Meso	Meso	Meso
Lys_Se_16	Eu	Meso	Meso-Eu	Eu	Eu	Veg_Se_87	Meso	Meso-Eu	Meso	Meso	Meso
MgP_Jn_16	Eu	Meso-Eu	Meso-Eu	Meso-Eu	Meso	Veg_Jn_17	Meso	Eu	Meso	Meso-Eu	Meso
MgP_Jl_16	Meso	Meso-Eu	Meso	Meso-Eu	Meso	Veg_Jl_17	Meso	Eu	Meso-Eu	Meso-Eu	Meso-Eu
MgP_Au_16	Meso	Meso-Eu	Meso	Meso-Eu	Meso	Veg_Au_17	Meso	Eu	Meso	Meso-Eu	Meso
MkP_Jn_90	Eu	Hyper	Meso-Eu	Meso-Eu	Meso-Eu	Vol_Jl_84	Eu	Eu	Meso	Meso-Eu	Meso
MkP_Jl_90	Eu	Hyper	Meso-Eu	Eu	Meso-Eu	Vol_Au_84	Eu	Eu	Meso	Meso-Eu	Meso-Eu
MkP_Au_90	Eu	Hyper	Meso-Eu	Eu	Meso-Eu	Vol_Se_84	Eu	Eu	Meso	Eu	Meso-Eu
MkP_Jl_91	Eu	Eu	Eu	Meso-Eu	Meso-Eu	Vol_Jl_85	Eu	Eu	Meso-Eu	Meso-Eu	Meso
MkP_Au_91	Eu	Eu	Meso	Eu	Meso-Eu	Vol_Au_85	Eu	Eu	Meso-Eu	Eu	Meso-Eu
MkP_Se_91	Eu	Eu	Meso-Eu	Eu	Meso-Eu	Vol_Se_85	Eu	Eu	Meso	Eu	Meso-Eu
MkP_Jl_92	Eu	Eu	Meso	Meso-Eu	Meso-Eu	Vol_Jn_86	Eu	Eu	Meso-Eu	Eu	Eu
MkP_Au_92	Eu	Eu	Meso	Meso-Eu	Meso-Eu	Vol_Jl_86	Eu	Eu	Meso-Eu	Eu	Eu
MkP_Se_92	Eu	Eu	Meso	Meso-Eu	Meso	Vol_Au_86	Eu	Eu	Meso-Eu	Eu	Eu
MkP_Jn_16	Eu	Meso	Meso-Eu	Meso-Eu	Meso	Vou_Jl_16	Hyper	Hyper	Meso-Eu	Meso-Eu	Meso-Eu
MkP_Jl_16	Meso	Meso	Meso	Meso-Eu	Meso	Vou_Au_16	Hyper	Hyper	Meso-Eu	Eu	Meso-Eu
MkP_Au_16	Eu	Meso	Meso-Eu	Meso-Eu	Meso-Eu	Vou_Se_16	Hyper	Hyper	Meso-Eu	Eu	Eu
Oze_Jl_16	Eu	Oligo	Meso-Eu	Meso	Meso-Eu	Yli_Jl_90		Hyper	Eu	Eu	Eu
Oze_Au_16	Meso	Oligo	Meso-Eu	Meso	Meso	Yli_Au_90	Hyper	Hyper	Meso-Eu	Eu	Meso-Eu
Oze_Se_16	Meso	Oligo	Meso-Eu	Meso	Meso-Eu	Yli_Se_90	Hyper	Hyper	Meso-Eu	Eu	Eu

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