VNIVERSITAT DE VALÈNCIA

What is this Thesis about?

Currently, worldwide most of freshwater resources are under several threats that negatively impact on water quality. To determine water quality within EU Water Framework Directive some groups of biota have been established, however, zooplankton was not considered. Employing a zooplankton database from a high number of Ebro`s watershed reservoirs. This thesis evaluates the use of zooplankton as water quality indicator under different approaches: taxonomic, abundances, biomass, functional groups and machine learning.

VNIVERSITAT DE VALÈNCIA

Facultad de Ciencias Biológicas

EL PAPEL DEL ZOOPLANCTON COMO INDICADOR DE CALIDAD DE AGUA APLICADO A LOS EMBALSES DE LA CUENCA DEL EBRO, ESPAÑA

Manuel Eduardo Muñoz Colmenares

Tesis Doctoral 2022

VNIVERSITAT DE VALÈNCIA

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THE ROLE OF ZOOPLANKTON AS INDICATOR OF WATER QUALITY APPLIED TO EBRO BASIN RESERVOIRS, SPAIN

EL PAPEL DEL ZOOPLANCTON COMO INDICADOR DE CALIDAD DE AGUA APLICADO A LOS EMBALSES DE LA CUENCA DEL EBRO, ESPAÑA

PhD THESIS **Manuel Eduardo Muñoz Colmenares**

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Dr. Juan Miguel Soria García

Doctoral Program in Pollution, Toxicology and Environmental Health
Programa de Doctorado en Contaminación, Toxicología y Sanidad Ambientales
November / Noviembre 2022

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PhD Thesis

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VNIVERSITAT D VALÈNCIA

CERTIFY that Mr. Manuel Eduardo Muñoz Colmenares was under our direction during his doctoral studies with high performance and during the elaboration of the present thesis to obtain his doctoral degree. And for the record, in compliance with current legislation, we issue this certificate and positive report in Valencia on October 31, 2022

CERTIFICAN que el sr. Manuel Eduardo Muñoz Colmenares estuvo bajo nuestra dirección durante sus estudios de doctorado con alto rendimiento y durante la elaboración de la presente tesis para obtener su grado doctoral. Y para que conste, en cumplimiento de la legislación vigente, emitimos este certificado e informe favorable en Valencia el 31 de octubre de 2022

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Thesis entitled:

"The role of zooplankton as indicator of water quality applied to Ebro basin reservoirs, Spain"

Tesis titulada:

"El papel del zooplancton como indicador de calidad de agua aplicado a los embalses de la cuenca del Ebro, España"

Submitted by Manuel Eduardo Muñoz Colmenares to obtain the degree of Doctor in Environmental Contamination, Toxicology and Sanitation

Presentada por Manuel Eduardo Muñoz Colmenares para obtener el grado de Doctor en contaminación, toxicología y sanidad ambientales.

Eduardo Vicente | Juan M Soria

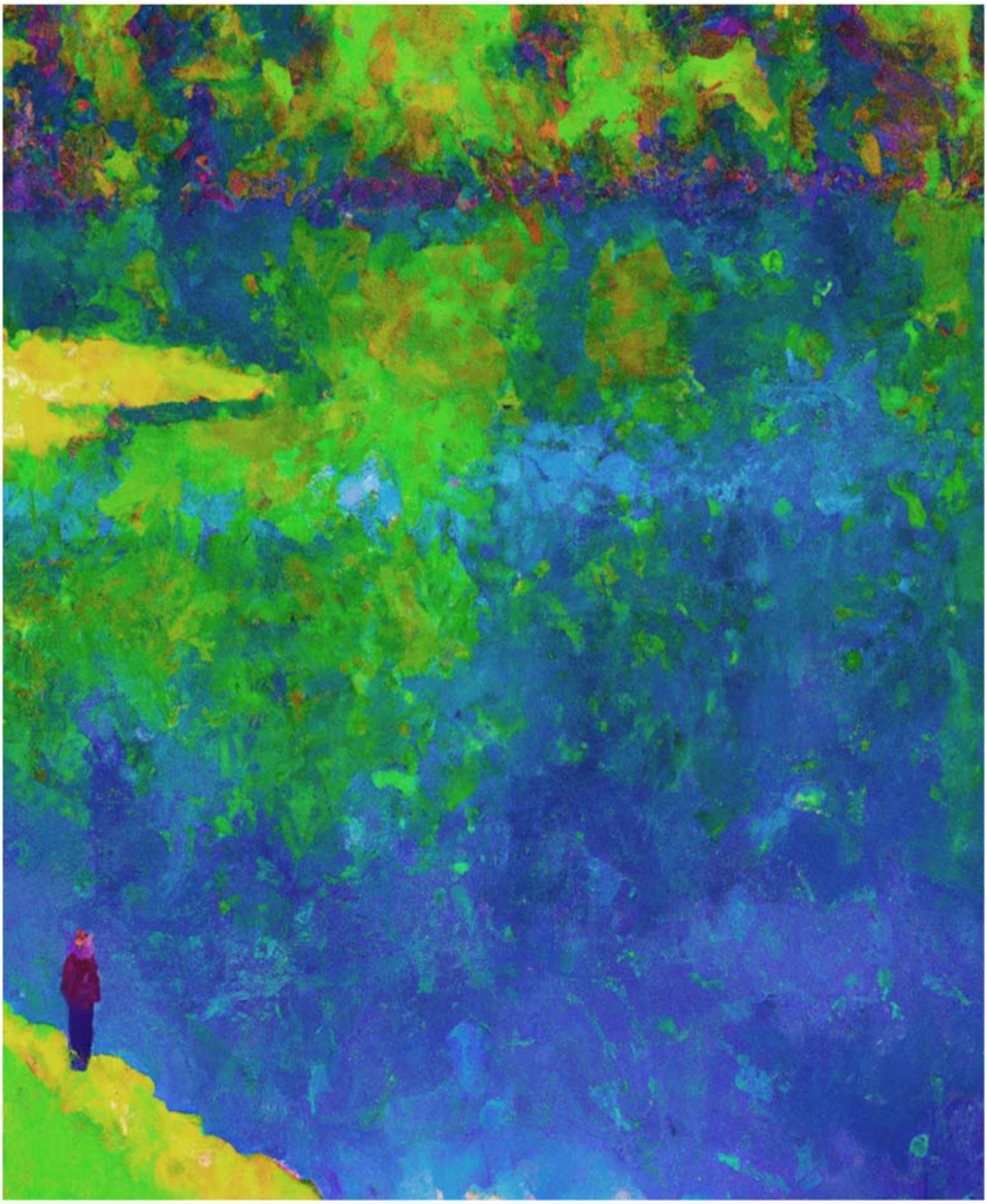
Dr. Eduardo Vicente Pedrós | Dr. Juan Miguel Soria García | Manuel E. Muñoz Colmenares

This thesis is dedicated to my mother

***In memoriam* Professor Maria Rosa Miracle**

"Born to lose, live to win"

Lemmy Kilmister



“Water quality”
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Bien se dice que el tiempo es relativo, justamente ahora ya han pasado cuatro años desde que empecé este viaje llamado la tesis doctoral, sin embargo, yo siento que ha sido poco el tiempo desde que lo inicie. Inclusive ese ahora ya lejano 2015 donde conocí por primera vez a la Dra. María Rosa Miracle (Q.E.P.D) me sigue pareciendo tan cercano. También aquel 2016 donde realicé una estancia académica en la Universidad de Valencia con la Dra. Rosa y donde conocí al Dr. Eduardo Vicente, lugar donde mi proyecto doctoral empezó a tomar forma.

Después de regresar y laborar durante dos años en México, pude conseguir una ansiada beca doctoral, la cual me pudo facilitar mi regreso a Valencia, así que agradezco al CONACYT al convertirme en un suertudote y otorgándomela. De la misma forma le doy las gracias a las pocas personas que estuvieron durante ese proceso, las cuales me soportaron durante los meses que duro la evaluación y me convertía una montaña rusa de emociones mientras esperaba resultados.

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Al iniciar tuve que dejar atrás a mi país y cambiarme de continente, adaptarme a otra cultura y aunque en el papel el idioma no fue una barrera, en algunas ocasiones por el uso de las palabras y costumbres se dieron muchos episodios de que yo o la otra persona no sabía que se había dicho, así que gracias a los que se tomaron el tiempo para dejarme explicar, el entenderme y explicarme las costumbres de tierras valencianas. Y no solamente las costumbres de estas tierras, si no, de todos aquellos países de los cuales fueron originarios las diferentes personas con las que compartí casa, viajes y aventuras.

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Durante mi tiempo académico conocí varios compañeros de laboratorio y doctorado, pero le doy gracias especialmente a Xavi y a Mariloli. Que durante mi primer año me hicieron sentir bienvenido en el departamento e integrarme de la mejor forma posible, además de ser un estupendo equipo de trabajo durante las campañas de muestreo en los embalses durante los cuatro años y tenerme paciencia durante los mismos. Patricia con la que hice una buena amistad dentro y fuera del ambiente laboral, ser mi gurú en temas burocráticos para las campañas y con la cual me puedo ir a reír de cosas random, compartir nuestra cultura latina y poder hablar con referencias de los Simpson. Rebe por no dejar que fuera la única alma en el laboratorio y compartir y entender todo lo que conlleva estar haciendo el doctorado, las pláticas y el hacerme ver algunas cosas desde otro punto de vista. Al Dani, que, aunque no compartimos mucho tiempo juntos congeniamos rápidamente y demostrar que es una gran persona. A Carla Ulmus y Mike Corrales que siempre han sido excelentes personas conmigo y ayudaron a integrarme un poco más en mi llegada a la ciudad.

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El compartir piso con gente desconocida puede llegar a ser muy complicado, sin embargo, tuve la buena fortuna de que siempre estuve con gente bastante agradable y de todos partes del mundo. Aquí agradezco haber podido compartir con mis italianas favoritas, Marika y Doriana. Que siempre alegraron mis días con sus ocurrencias, personalidades, su cocina italiana, pero sobre todo con su amistad. También a mi vuelta de Oporto el poder compartir con la Dra. Joanna (Asia) Rosińska, "the best roomie", por el siempre hacerse un espacio para poder hablar, contarme su experiencia en la investigación, sus grandes comentarios y revisiones tanto en mi último artículo como en la presente tesis. Por ser alguien tan inteligente y genial, ¡gracias!

El tener un buen balance dentro de la vida diaria es importante, y hay personas que simplemente con su presencia te ayudan. Por lo cual agradezco a Valeria, por compartir su tiempo conmigo, el escucharme, entenderme, aconsejarme y tranquilizarme en mis casi siempre momentos de estrés del último año. El también el dejarme conocerla más como es en realidad, el enseñarme de su cultura, en decirme sus miedos y esperanzas y el que sea autentica conmigo, Люблю тебя Дорогая.

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Index

Abstract

Resumen

Abstract

Nowadays, freshwater resources are under several pressures and threats, mostly resulting from human activities. These threats can promote an accelerate eutrophication process inside waterbodies. An accelerated eutrophication can impact negatively water quality and affect the populations of many different species that inhabit freshwater environments or that are relate to them. Also, a reduced water quality can jeopardize the availability of water resources for human activities and necessities. To address this situation, the so-called EU Water Framework Directive (WFD: Directive 2000/60/EC) was established. Inside this directive were included several groups as water quality indicator, although zooplankton was not included. Zooplankton communities have a fundamental ecological role in aquatic food webs. They contribute significantly to nutrient recycling and are the connector in the energy transfer between primary producers and higher trophic levels. Furthermore, zooplankton community can control phytoplankton blooms and are highly sensitive to environmental changes.

The current thesis was elaborated as a response to the exclusion of zooplankton as a Biological Quality Element within the WFD. The main aim was to evaluate whether zooplankton communities are good indicators to determine water quality of targeted waterbodies, and, in particular to determine trophic status and ecological potential. To achieve this task different approaches were used: i) determine which environmental variables are associate to zooplankton community ii) identify the species associated to good and low water quality, iii) determine if zooplankton abundance and biomass could differentiate among water quality levels, iv) determine the functional groups presents within reservoirs and indicating which could be used as indicator besides the implementation of machine learning. In this thesis, we presented a high representative data from zooplankton communities that inhabit in more than 60 reservoirs located in the largest Spain watershed, the Ebro basin. From all those we obtained

more than 300 sampling occasions or samples during the summer season in the period 2010 - 2019.

Zooplankton metacommunity was composed of 169 species: 115 species of rotifers, 36 of cladocerans and 17 species of copepods. Also was detected the presence of zebra mussel invader *Dresseina polymorpha* in several reservoirs. This mussel was detected for first time at La Sotonera reservoir. Zooplankton groups were associated with several environmental variables. Microcrustaceans were mainly related to those variables that determine trophic status and ecological potential such as, chlorophyll a, total phosphorus, dissolved oxygen and Secchi disk transparency. Several indicator species for water quality were found. Species considered as indicators of good water quality (oligotrophy and good or higher ecological status) were *Daphnia longispina*, *Ascomorpha ovalis* and *Ascomorpha saltans*. An indicator of moderate quality was *Bosmina longirostris,* while indicators species of bad water quality were *Acanthocyclops americanus*, *Ceriodaphnia* spp., *Daphnia cucullata*, *Daphnia parvula*, *Diaphanosoma brachyurum, Brachionus angularis, Keratella cochlearis* and *Pompholyx sulcata*.

The proposed metrics based on zooplankton abundance and biomass with better sensitivity were ZOO (total zooplankton), LZOO (large zooplankton), CLAD (cladocerans), and ZOO:CHLA (zooplankton:chlorophyll a ratio). Most of microcrustacean metrics at group or genera level were good at differentiating between high and low water quality in trophic status (oligotrophic–eutrophic) and ecological potential (good or higher–moderate).

Using a functional approach, five functional groups were identified: large filter copepods, raptorial copepods, cladocerans, microphagous rotifers and raptorial rotifers. Low densities of large filter-feeding groups like calanoid copepods and cladocerans were indicators of good water quality. In contrast, high raptorial cyclopoid copepods abundances served as indicators of low water quality. Using a Random Forest as a machine learning approach, several predictive models were obtained, which accuracy varied from 41% to 77% among functional groups and water quality levels respectively. Finally, using Classification and Regression Trees, thresholds were estimated to determine water quality. In the case of the trophic status and ecological potential, thresholds to differentiate good from bad water quality were somehow similar, thus are proposed as a guide the following thresholds: calanoids <1.4 ind. L^{-1}, raptorial cyclopoids <0.38 ind. L^{-1} and cladocerans <19.36 ind. L^{-1}.

The results from this thesis indicates that zooplankton is a useful water quality indicator. I have shown that it is possible to determine trophic status and ecological potential using zooplankton community under different approaches. This thesis contributed to increase the knowledge of zooplankton and its use as a water quality indicator. Lastly, I suggest that zooplankton community should be incorporate as a Biological Quality Element within the Water Framework Directive.

Resumen

En la actualidad, los recursos de agua dulce están sometidos a diversas presiones y amenazas, una gran parte de estas se debe a diversas actividades humanas. Estas amenazas pueden ocasionar un acelerado proceso de eutrofización dentro de los cuerpos de agua. Una eutrofización acelerada puede repercutir negativamente en la calidad del agua, haciendo que esta disminuya. Lo cual afecta a las comunidades y poblaciones de muchas especies diferentes que viven en los cuerpos de agua o se relacionan con ellos. Además, una mala calidad del agua puede poner en peligro la disponibilidad de recursos hídricos para las actividades y necesidades humanas. Para hacer frente a esta situación, la unión europea estableció la Directiva Marco del Agua (DMA). Dentro de esta directiva se incluyeron varios grupos acuáticos como indicadores de la calidad del agua, sin embargo, y sorpresivamente el zooplancton no fue incluido. La comunidad del zooplancton tiene un papel ecológico fundamental en la transferencia de energía dentro de las cadenas tróficas acuáticas. Esta comunidad contribuye significativamente al reciclaje de nutrientes y son el conector o eslabón en la transferencia de energía entre los productores primarios y consumidores secundarios. Por otro lado, la comunidad de zooplancton puede controlar las floraciones de microalgas y posee una gran sensibilidad a los cambios del entorno.

La presente tesis se elaboró como una respuesta de investigación a la exclusión de la comunidad de zooplancton como un elemento de calidad biológica dentro de la DMA. El objetivo principal de esta tesis es evaluar si las comunidades de zooplancton son buenos indicadores para determinar la calidad del agua. En específico, para determinar el estado trófico y el potencial ecológico. Para lograr esto y como objetivos particulares en los diferentes capítulos utilizamos diferentes enfoques: i) determinar qué variables ambientales están asociadas a la comunidad de zooplancton ii) identificar las especies asociadas a la buena y a la baja calidad del agua, iii) determinar si la abundancia y la biomasa podrían diferenciar entre los

niveles de calidad del agua esto a través ciertas métricas propuestas, iv) determinar los grupos funcionales presentes dentro de los embalses e indicar cuáles podrían ser utilizados como indicador además de la implementación del aprendizaje automático. En esta tesis, presentamos datos altamente representativos de la comunidad de zooplancton que habita en más de 60 embalses situados en la cuenca del Ebro, la cual es la mayor cuenca hidrográfica de España. De todos ellos obtuvimos más de 300 ocasiones de muestreo o muestras durante la temporada de verano durante el periodo de 2010 a 2019. En el primer capítulo, como un análisis prospectivo se utilizaron solo los datos de seis embalses durante las temporadas de verano y otoño. Mientras que los tres restantes capítulos se utilizaron los datos de todos los embalses y muestras.

La comunidad de zooplancton estuvo compuesta por 169 especies en total. Los rotíferos contaron con 115 especies, mientras que los cladóceros y copépodos estuvieron representados con 36 y 17 especies respectivamente. También se detectó la presencia invasora del mejillón cebra *Dresseina polymorpha* en varios embalses a lo largo de la cuenca. Esta especie invasora se detectó en el embalse de La Sotonera, el cual se había reportado tiempo atrás libre de este mejillón. Los diferentes grupos de zooplancton estuvieron relacionados con diversas variables ambientales. Los microcrustáceos se relacionaron principalmente con las variables que determinan el estado trófico y el potencial ecológico tales como la clorofila a, el fósforo total, el oxígeno disuelto y la transparencia del disco de Secchi. Con un acercamiento taxonómico se identificaron diversas especies indicadoras de la calidad del agua. Las especies consideradas como indicadores de buena calidad del agua (oligotrofia y estado ecológico bueno o superior) fueron *Daphnia longispina, Ascomorpha ovalis* y *Ascomorpha saltans.* El cladocero *Bosmina longirostris* fue catalogado como un indicador de calidad moderada. Mientras que las especies indicadoras de mala calidad del agua fueron *Acanthocyclops americanus, Ceriodaphnia spp., Daphnia cucullata, Daphnia parvula, Diaphanosoma brachyurum, Brachionus angularis, Keratella cochlearis* y *Pompholyx sulcata*

Las métricas propuestas basadas en la abundancia y biomasa del zooplancton con mejor sensibilidad para diferenciar entre la calidad de agua fueron ZOO (zooplancton total), LZOO (zooplancton grande), CLAD (cladóceros) y ZOO:CHLA (relación zooplancton:clorofila a). La mayoría de las métricas de los microcrustáceos a nivel de grupo o de género fueron buenas para diferenciar entre la calidad del agua alta y baja en cuanto al estado trófico (oligotrófico-eutrófico) y el potencial ecológico (bueno o superior-moderado). Utilizando un enfoque funcional identificamos cinco grupos funcionales, los cuales fueron: copépodos filtradores, copépodos rapaces, cladóceros, rotíferos microfagos y rotíferos rapaces. Con este enfoque funcional observamos que las bajas densidades de los grandes grupos filtradores, como los copépodos calanoides y los cladóceros, eran indicadores de una buena calidad del agua. Por el contrario, la alta abundancia de copépodos ciclopoideos sirvió como indicador de una baja calidad del agua. Empleando la técnica de Bosques Aleatorios como enfoque de aprendizaje automático, obtuvimos varios modelos de predicción, cuya precisión variaba del 41% al 77% entre los grupos funcionales y los niveles de calidad del agua. Finalmente, utilizando árboles de clasificación y regresión estimamos umbrales para determinar la calidad del agua. En el caso del estado trófico y del potencial ecológico, los umbrales para diferenciar la buena de la mala calidad del agua fueron en cierto modo similares, por lo que proponemos como guía los siguientes umbrales: copépodos calanoides <1,4 ind. L^{-1}, ciclopoides rapaces <0,38 ind. L^{-1} y cladóceros <19,36 ind. L^{-1}.

Finalmente, los resultados de esta tesis indican que el zooplancton es un buen indicador de la calidad del agua. Esto, ya que fue posible determinar el estado trófico y el potencial ecológico utilizando la comunidad de zooplancton bajo diferentes enfoques. Esta tesis ha contribuido a aumentar el conocimiento del zooplancton y su uso como indicador de la calidad del agua. Por último, sugerimos que la comunidad del zooplancton sea incorporada como un elemento de calidad biológica dentro de la Directiva Marco del Agua.

Normative/ Normativa

This report summarizes the work performed by the doctoral student between November 2018 and November 2022 in the Department of Microbiology and Ecology, Faculty of Biology. University of Valencia.

All the legal requirements to obtain the degree of Doctor by the University of València are now presented in Spanish, one of the official languages of this university. Particularly, they refer to the requirements to conduct a PhD by publications and to obtain an International PhD. In this sense, the thesis is written in English and part of the research was performed abroad: a three-moth stay in Portugal under the supervision of Dr. Sara Antunes at the University of Porto, Portugal.

Esta memoria resume el trabajo realizado por el doctorando entre noviembre de 2018 y noviembre de 2022 en el Departamento de Microbiología y Ecología de la Facultad de Biología. Universidad de Valencia.

Todos los requisitos legales para obtener el título de Doctor por la Universitat de València se presentan ahora en castellano, una de las lenguas oficiales de esta universidad. En particular, se refieren a los requisitos para realizar un doctorado por publicaciones y para obtener un doctorado internacional. En este sentido, la tesis está redactada en inglés y parte de la investigación se realizó en el extranjero: una estancia de tres meses en Portugal bajo la supervisión de la Dra. Sara Antunes en la Universidad de Oporto, Portugal.

Instrucción tomada de

https://www.uv.es/doctor/Normativa/Instruccion_mencion_internacional_ED.pdf
fecha de consulta: 7 de julio de 2022:

"INSTRUCCIÓN PARA LA OBTENCIÓN DE LA MENCIÓN INTERNACIONAL DEL TÍTULO DE
DOCTOR, APROBADA POR LA JUNTA PERMANENTE DE LA ESCUELA DE DOCTORADO EL 13 DE JULIO DE 2021
(Modificada por acuerdo de la Junta Permanente de la Escuela de Doctorado de 3 de mayo de 2022)
1. Se puede otorgar la mención internacional al título de doctor, siempre que concurran las circunstancias siguientes:

a) Que, durante el periodo de formación necesario para obtener el título de doctor, el doctorando o la doctoranda haya realizado una estancia mínima de tres meses en una institución de enseñanza superior o centro de investigación de prestigio fuera de España, suficientemente acreditada y con financiación a través de convocatorias competitivas para estancias cortas, en las que haya cursado estudios o haya realizados trabajos de investigación. La estancia y las actividades deberán haber sido avaladas por el director o la directora y autorizadas por la comisión académica del programa de doctorado, y será necesario que se incorporen al documento de actividades del doctorando o la doctoranda. En el supuesto de que el doctorando o doctoranda haya solicitado financiación y no se le haya concedido, se remitirá a la Escuela de Doctorado para su aprobación, con una antelación mínima de un mes antes de la estancia, junto con el informe favorable de la comisión académica, el CV de la persona responsable de la estancia, el del director o directora del equipo receptor y el plan de trabajo que hay que llevar a cabo; la documentación se presentará como mínimo un mes antes del inicio de la estancia; no se podrá comenzar la estancia sin la autorización previa de la Escuela de Doctorado. En el caso de que la estancia no disponga de financiación competitiva, se deberá informar a la comisión de coordinación académica del programa de doctorado correspondiente en un plazo previo a su realización no inferior a tres meses. En caso de estancia a realizar en más de un

periodo, los periodos deberán tener una duración mínima ininterrumpida de un mes y como máximo se podrá fragmentar en dos periodos. En cualquier caso, el informe deberá incluir la planificación y justificación de la totalidad de los periodos previstos (duración, actividades previstas a realizar) para completar una estancia mínima de tres meses. La comisión de coordinación académica del programa de doctorado correspondiente será la responsable de informar, en su caso, la estancia, y lo comunicará a la Escuela de Doctorado, que dispondrá de quince días para solicitar, si fuera necesario, las aclaraciones oportunas. La subcomisión del área pertinente de la Escuela de Doctorado emitirá el informe preceptivo y autorizará, si procede, dicha estancia. En todo caso, el formato de solicitud de estancia debe incluir el CV de la persona responsable de la estancia, el del director o directora del equipo receptor y el plan de trabajo que hay que llevar a cabo.
b) Que parte de la tesis doctoral, al menos el resumen y las conclusiones, se haya redactado y sea defendida en una de las lenguas habituales para la comunicación científica en su campo de conocimiento, diferente de cualquiera de las lenguas oficiales en España, sin perjuicio del artículo 7.2. En ese caso, el resumen deberá tener una extensión mínima de 15.000 palabras. La comisión de coordinación académica de cada programa de Doctorado podrá pedir, previa justificación, que la mayor parte de la tesis o su totalidad sea redactada o sea defendida en una lengua extranjera. Este apartado no es aplicable para las estancias, los informes y los expertos que procedan de un país de habla hispana.
c) Que hayan informado positivamente sobre la tesis, previamente a la lectura, un mínimo de dos expertos o expertas con experiencia investigadora acreditada que pertenezcan a alguna institución de educación superior o instituto de investigación no españoles. Dichos expertos no pueden ser los responsables de la estancia mencionada en el apartado a), ni formar parte del equipo de trabajo de la investigación llevada a cabo en dicha estancia. El director o la directora deberá

aportar un escrito justificando la adecuación de los dos expertos o expertas internacionales propuestos.

d) Que haya formado parte del tribunal evaluador de la tesis un experto o experta que pertenezca a alguna institución de educación superior o centro de investigación no españoles, con vinculación estable, igual o superior a 5 años, con el título de doctor y con experiencia investigadora acreditada, siempre que no sea el responsable de la estancia mencionada en el apartado a).

2. La defensa de la tesis tiene que realizarse en la universidad donde el doctorando o la doctoranda esté adscrito/a. El doctorando o la doctoranda que quiera solicitar la concesión de la mención de "doctor internacional", ha de hacer constar esta circunstancia a la hora de pedir la autorización de depósito de la tesis doctoral."

3. Tanto en el caso de tesis bajo co-tutela como en aquellos sin co-tutela, será necesario realizar una estancia de tres meses en una universidad/centro diferente a las de afiliación de los/as directores/as, para poder obtener la mención internacional.

4. Las estancias en la universidad de origen del estudiante no serán consideradas válidas parala mención internacional.

Author contribution to papers

This PhD thesis is based on four original papers, organized and presented in the Chapters section. The contribution of the author to those publications was as follows:

Chapter 1. Muñoz-Colmenares, M. E., Vicente, E., Soria, J.M, and M. R. Miracle. 2021. **Zooplankton changes at six reservoirs in the Ebro watershed, Spain**. *Limnetica*. 40(2), 279-294. https://doi.org/10.23818/limn.40.19. EV, MRM and JMS planned the field campaigns. All four author were on samples collection. MEMC performed zooplankton counting and data analysis. MEMC wrote the paper draft. EV and JMS improve the paper with reviews, modifications and suggestions.

Chapter 2. Muñoz-Colmenares, M.E., Soria, J.M., and Vicente E. 2021. **Can zooplankton species be used as indicators of trophic status and ecological potential of reservoirs**? *Aquatic Ecology*, 55, 1143–1156. https://doi.org/10.1007/s10452-021-09897-8. EV and JMS designed the field sampling campaigns. All three authors were present during field works. MEMC designed the manuscript and performed species identification. MEMC performed the data analyses and wrote the paper draft. JMS and EV reviewed the data analyses and improved the final manuscript.

Chapter 3. Muñoz-Colmenares, M.E.; Sendra, M.D.; Sòria-Perpinyà, X.; Soria, J.M.; Vicente, E. 2021. **The use of zooplankton metrics to determine the trophic status and ecological potential: an approach in a large Mediterranean watershed**. *Water*, 13, 2382. https://doi.org/10.3390/w13172382. EV and JMS designed the field sampling campaigns. All five authors were present during different field campaigns. SMD counted and identified phytoplankton samples. MEMC performed species identification, data analyses and wrote the manuscript. JMS and EV reviewed and improved the paper before journal submission.

Chapter 4. Muñoz-Colmenares, M.E., Soria, J.M., and Vicente E. 2022. **Zooplankton functional groups and machine learning to determine water quality in reservoirs.** Submitted to *Inland Waters.* EV and JMS designed the field sampling campaigns. All three authors were present during different field campaigns. MEMC designed the manuscript. MCME performed the data analyses and wrote the manuscript draft. JMS and EV reviewed and improved the final manuscript.

Resumen en extenso

Versión en español

Resumen en extenso[1]

[1]Este es un resumen en extenso sin referencias, tablas ni figuras donde se resume la temática global de la tesis, se establecen los objetivos, se repasa la metodología empleada y se presentan y discuten los principales resultados. Por último, se muestran las consideraciones finales y conclusiones.

Introducción

Los sistemas acuáticos y la problemática a la que se enfrentan

El agua es un elemento fundamental para la vida, de esta depende el desarrollo tanto de los organismos como el de los ecosistemas. Aproximadamente el 97% del agua en el planeta contiene altas cantidades de sal, esta se encuentra en los mares y océano. Mientras que solamente alrededor del 3% contiene muy bajas cantidades o nada de sal, la cual es denominada como agua dulce. Poco más de la mitad del agua dulce se encuentra en estado sólido y se localiza en los polos, mientras que el restante se encuentra dentro de los diferentes continentes y son llamadas aguas epicontinentales. Estas aguas se dividen principalmente en aguas superficiales y subterráneas. Dentro de las primeras podemos encontrar aguas naturales como ríos y lagos, y las modificadas como es el caso de los embalses.

Actualmente, todos los cuerpos de agua se encuentran amenazados o bajo diferentes grados de presión. La principal amenaza se debe a las actividades humanas y a su necesidad de uso del recurso hídrico. Esta necesidad puede provocar una sobreexplotación, además de que puede provocar otros problemas como una mala gestión hídrica, eutrofización, contaminación y con ello una pérdida global de la calidad del medio acuático. Por otra parte, los efectos del cambio global sobre el planeta también impactan en los cuerpos de agua y su calidad, esto puede ser debido al incremento de las temperaturas y las consecuencias dimanantes de ello.

La eutrofización se debe a un aumento en la cantidad de nutrientes, que están recibiendo los cuerpos de agua a lo largo del tiempo, estos nutrientes son principalmente fosforo y nitrógeno. La eutrofización puede ser natural, que ocurre a través de un largo periodo de tiempo o puede ser debido a la actividad humana, la cual ocurre en poco tiempo. Este tipo de eutrofización también es llamada eutrofización antropogénica. Esta puede ser por diferentes desechos, fertilizantes o materia orgánica vertidos en un cuerpo de agua. Inclusive, aunque estos no sean vertidos directamente a un lago, por ejemplo, pueden llegar a través del lavado que se hace dentro de una cuenca hidrológica al llover, y terminar dentro de un sistema acuático. Este incremento de nutrientes puede afectar al sistema y puede facilitar la aparición de algas verdeazuladas o cianobacterias. Estas pueden producir toxinas, las cuales pueden afectar al resto de comunidades que habitan en el ecosistema de diferentes formas, por otro lado, estas cianotoxinas también pueden poner en riesgo a la salud humana. Este aumento de nutrientes no solo puede promover la aparición de las cianobacterias, sí que no, también puede incrementar la abundancia y biomasa de las microalgas, este aumento puede impactar negativamente en la calidad del agua.

El calentamiento global afecta de variadas formas a los cuerpos de agua. Por ejemplo, se ha detectado que el tiempo que los lagos que normalmente se encuentran congelados durante el invierno se ha visto reducido y se ha hecho más delgada la capa de hielo de los mismos. Mientras que, en lagos donde no existe durante ninguna temporada capa de hielo, ha favorecido a una mayor y más prolongada estratificación, incluso en aquellos sistemas polimícticos. El cambio climático al tener temperaturas y temporadas más extremas, en conjunto con sequias más largas, puede favorecer la desaparición de cuerpos de agua someros que antes se podían encontrar todo el año. Pasando así a volverse a cuerpos de agua temporales, volviendo únicamente en las temporadas de lluvias abundantes.

La acelerada eutrofización por las actividades humanas, el calentamiento global, la contaminación, entre otros, tienen impactos y consecuencias ecológicas en los sistemas acuáticos. Tales impactos pueden ser cambios en las cadenas tróficas y dentro de las comunidades que los habitan. Por ejemplo, disminuyendo factores como la calidad del agua, la capacidad productiva de aquellos cuerpos de agua que son utilizados en la pesca y acuacultura y la diversidad de especies tales como aves, mamíferos, peces y organismos microscópicos.

Dentro de las comunidades que habitan los diferentes sistemas acuáticos, existe una compuesta por organismos que viven suspendidos en la columna de agua, que no pueden nadar en contra de la corriente y que son independientes del fondo. Para este tipo de organismos se les acuño el término "plancton". El plancton es pequeño y generalmente no es más grande que 5 milímetros (mm) y dependiendo de su tamaño se le puede clasificar en diferentes categorías. Femtoplancton compuesto por virus y con un tamaño menor a 0.2 µm. Picoplancton de 0.2 a 2 µm y formado principalmente por bacterias. Nanoplancton entre 2 y 20 µm formado por autótrofos y heterótrofos como flagelados, ciliados y pequeñas algas. El microplancton (20-200 µm), el mesoplancton (0.2 – 2 mm) y macroplancton (mayor de 2 mm) son representados principalmente por microalgas y diferentes grupos de heterótrofos como rotíferos, microcrustáceos y larvas de diferentes grupos, como peces, cnidarios, equinodermos entre otros.

El plancton está conformado por dos grandes grupos, el primero es el fitoplancton. Este grupo está compuesto por algas unicelulares que pueden vivir independientes o formando colonias, estas algas son organismos autótrofos y obtienen su energía mediante el proceso fotosintético. Algunas de las clases más representativas del fitoplancton son: *Chlorophyceae* (algas verdes), *Bacillariophyceae* (diatomeas), *Dinophyceae* (dinoflagelados), *Cyanophyceae* (algas verdeazuladas), *Phaeophyceae* (algas pardas) y *Rhodophyceae* (algas rojas).

Al fitoplancton se le considera como la unidad básica de producción de la materia orgánica o también considerados como el productor primario dentro de la cadena de energía en los ecosistemas acuáticos, ya que son la principal fuente de energía del siguiente nivel, y el cual es también el segundo grupo del plancton: el zooplancton.

¿Qué es y quienes son el zooplancton?

El zooplancton está compuesto por una gran diversidad de organismos heterótrofos de diferentes filos y clases de animales invertebrados. El zooplancton a diferencia del fitoplancton puede estar conformado por animales que toda su vida no pueden nadar contra corriente o ser solo una etapa larval de organismos que después no cumplirán esa regla, como los peces. Los grupos que conforman el zooplancton dependen también en gran medida del medio en que se encuentre, siendo diferentes entre el marino y el de agua dulce. En el caso de este último, el zooplancton de agua dulce está constituido principalmente por tres grandes grupos: los rotíferos, los cladóceros y los copépodos.

Los rotíferos, son organismos pseudocelomados con un tamaño aproximado comprendido entre 50 a 2000 µm y poseen una simetría bilateral. Los rotíferos también presentan una característica peculiar llamada eutelia, la cual consiste en que una especie siempre presentará el mismo número de células al llegar a la madurez siendo constante para los organismos de la misma especie. Hasta el momento se han descrito más de 2000 especies diferentes y se tienen registros de estos animales en prácticamente todos los ambientes acuáticos, e inclusive en ambientes terrestres húmedos; sobre líquenes y musgos. A este grupo lo definen tres características principales, primero, que en la parte anterior poseen una corona ciliada que les sirve para filtrar su alimento, así como de locomoción. Segundo, la presencia de un mastax o trophi en la parte interior del cuerpo para procesar el alimento ingerido o capturado y, por último, el cuerpo puede o no puede estar loricado (suave o protegido con cubierta dura). La estrategia reproductiva de los rotíferos puede ser sexual o asexual, siendo esta ultima la que se da generalmente por medio de la partenogénesis.

Junto a los rotíferos, los microcrustáceos dominan los cuerpos de agua dulce. El primer grupo de los microcrustáceos lo conforman los cladóceros, los cuales hasta el momento se han descrito más de 600 especies, aunque se estima que este número puede ser mucho más alto. Presentan un cuerpo blando que se encuentra rodeado por dos valvas hechas de quitina y su tamaño generalmente comprende entre los 0.2 a los 3 mm, aunque una especie puede alcanzar hasta los 18 mm. El cuerpo blando de los cladóceros presenta de 4 a 6 pares de apéndices torácicos, los cuales tienen la función principal para la selección y filtración del alimento. Poseen una cabeza, que también se encuentra protegida por quitina, en ella presentan un ocelo, dos ojos y sobresalen dos pares de apéndices; las anténulas y las antenas. En la parte final del cuerpo, poseen un postabdomen que puede usarse como locomoción o para la limpieza de los apéndices filtradores. Al igual que los rotíferos, presentan reproducción sexual y asexual por medio de reproducción partenogenética, siendo la asexual la que prevalece.

Los copépodos son el segundo grupo de microcrustáceos y el tercer grupo que domina el zooplancton, además de ser los de mayor tamaño, ya que van desde los 0.5 hasta 5 mm en su forma adulta. El cuerpo presenta una forma cilíndrica y segmentada, la parte de la cabeza presenta un ojo simple y un par de antenas y anténulas. El tórax presenta diferentes pares de apéndices mientras que la parte posterior, presenta un rami que cuenta con dos setas caudales. A diferencia de los otros dos grupos, este grupo solo presenta reproducción sexual con la producción y fertilización de huevos. A lo largo de su vida pasan a través de diferentes etapas, siendo la primera la forma nauplio, para posteriormente ser copepoditos y finalmente su forma adulta. Son el grupo con una mayor diversidad en cuanto a número de especies. En los ambientes marinos se han identificado más de 10,000 especies, mientras que en aguas dulces se han descrito más de 1,500. Dentro de las especies que habitan en agua dulce, la gran mayoría pertenecen a tres órdenes, Calanoidea, Cyclopoidea y Harpaticoidea.

Por último, en determinados ambientes de agua dulce, pueden formar parte del zooplancton ciertos grupos zoológicos en su forma larvaria, que podrían llegar a dominar el plancton. Uno de estos ejemplos es el de las larvas velígeras del mejillón cebra, la cual pueden tener una abundancia y biomasa muy alta en los cuerpos de agua en comparación con el resto de las comunidades planctónicas. Esto se puede ver en diferentes regiones, como es el caso de los embalses en la cuenca del Ebro. En el caso de los ambientes de los ambientes de agua salada, diferentes grupos como larvas trocóforas y larvas de peces pueden ser más dominantes bajo ciertas condiciones que los microcrustáceos.

El rol del zooplancton en los ambientes acuáticos

El papel que desempeña el zooplancton dentro de los ecosistemas acuáticos es de gran importancia y puede ser de forma variada. El zooplancton dentro de la cadena trófica facilita el flujo de energía entre los productores primarios como lo son las algas y los consumidores secundarios como los peces. Una forma bastante común de hacer una simplificación de la cadena de energía es de la siguiente forma: fitoplancton, zooplancton, pequeños peces planctívoros y peces piscívoros. Sin embargo, hay muchos más elementos involucrados, como es el caso del detritus que puede llegar a ser más dominante que el fitoplancton cuando este no tenga la biomasa suficiente dentro de un ambiente. Al tener más disponibilidad de detritus, favorecería a las especies consumidoras del mismo de los siguientes niveles. También se encuentran las bacterias, ciliados y flagelados heterotróficos del bucle microbiano, que también pueden llegar a ser una gran fuente de energía. Debido en parte a que algunas especies del zooplancton que pueden llegar a filtrarlas y consumir a estos organismos, así teniendo interacción con el bucle microbiano e integrándolo a la cadena trófica en un nivel más alto.

Aunque se menciona que el siguiente nivel que consume al zooplancton son los peces, estos no son sus únicos depredadores. Ya que también pueden llegar a ser alimento de insectos acuáticos y otras especies depredadoras pertenecientes al mismo zooplancton, como lo son los copépodos ciclopoideos, varios géneros de rotíferos y el cladocero *Leptodora kindtii.* Estos depredadores dentro del mismo nivel hacen que la energía y el carbón no se mueva exclusivamente de forma lineal dentro de la cadena trófica, resaltando la importancia de las especies del zooplancton en ella.

Otra función que tiene el zooplancton es su rol dentro del control de arriba abajo (top-down control). Este control indica que el zooplancton ejerce una presión sobre el fitoplancton, lo cual moldeará la estructura y la dinámica de la comunidad fitoplanctónica. Por ejemplo, al aumentar la abundancia de las comunidades del zooplancton filtradoras de algas, la presión sobre el fitoplancton será mayor, provocando que la densidad algal disminuya en aquellas especies que pueden ser consumidas. Esto puede disminuir la diversidad algal y también puede tener como resultado una columna de agua más transparente. En caso contrario, durante un evento de control de abajo hacia arriba (bottom up), el zooplancton puede ser afectado debido a la densidad fitoplanctónica. Por ejemplo, durante el invierno la densidad fitoplanctónica disminuye, haciendo que las densidades del zooplancton también lo hagan. En caso contrario, el zooplancton puede ser afectado por un Bloom o un gran incremento en la densidad fitoplanctónica. En este caso, el zooplancton también puede responder rápidamente a este incremento y aumentar su densidad poco tiempo después del aumento fitoplanctónico. Si no se controlara el gran incremento del fitoplancton, este podría ocasionar entre otras cosas, que la turbidez del agua sea mayor afectando a otras especies. Por lo cual, el proceso de controlar al fitoplancton y como medio de la transferencia de energía hacen que el zooplancton sea un elemento clave dentro de los sistemas acuáticos.

La DMA y la exclusión del zooplancton

Debido a la problemática y a la amenaza que enfrentan gran parte de los cuerpos de agua mundialmente, se han tomado ciertas medidas en diferentes lugares. En el caso de la Unión Europea, se implementó dentro de su política de gestión de aguas la Directiva Marco del Agua (DMA) en octubre de 2000. Esta directiva presenta y establece las pautas metodológicas dentro del marco comunitario a seguir para la clasificación del estado ecológico de sus cuerpos de agua. El mayor objetivo de la DMA es el alcanzar un buen estado ecológico de las masas de agua superficiales de sus estados miembros.

Para hacer una buena estimación del estado ecológico, la DMA establece las diferentes categorías en las que se pueden clasificar los cuerpos de agua con respecto a su estado o potencial ecológico. Esta clasificación se divide de la siguiente manera de mejor a peor estado: Bueno o Superior, Moderado, Deficiente y Malo. Para llegar a estas clasificaciones se miden diferentes variables, que pueden ser fisicoquímicas y/o biológicas. En el caso de las variables fisicoquímicas, se toman en cuenta las siguientes: la transparencia medida por medio del disco de Secchi, la concentración de oxígeno disuelto y la concentración de nutrientes medida a través del fósforo total. Estas variables tienen una alta relación con la productividad biológica, la capacidad del sistema para asimilar la materia orgánica y la alta cantidad de nutrientes pueden ocasionar procesos de eutrofización al ser el fosforo el elemento limitante para las algas.

Se denominaron Elementos de Calidad Biológica (ECB) a los indicadores biológicos que fueron seleccionados siguiendo los nuevos requerimientos de la DMA y que son mencionados en su anexo V. Estos ECB poseen diferentes estrategias, ciclos de vida y sensibilidades a los cambios que se producen en el medio acuático. Las comunidades que conforman a estos ECB fueron: los peces, los macroinvertebrados, las plantas macrófitas, el fitoplancton y el fitobentos. Sin embargo, para sorpresa de muchos, el zooplancton no fue incluido dentro de esta lista.

La omisión del zooplancton dentro de la DMA puede ser argumentada debido a que las relaciones del zooplancton con los procesos de eutrofización no habían sido suficientemente estudiadas durante el siglo XX y no son tan directas como los son con el fitoplancton. Sin embargo, como se mencionó anteriormente, este elemento es un componente muy importante y clave que pueden moldear las comunidades tanto de los niveles inferiores como superiores en la cadena trófica.

Por otro lado, a lo largo de diferentes estudios alrededor del mundo se ha podido establecer que las comunidades que conforman al zooplancton son particularmente sensibles a los cambios en el ambiente donde habitan. La mayoría de estos estudios se han centrado sobre los tres grandes grupos del zooplancton que fueron mencionados con anterioridad. Se ha encontrado que hay especies o grupos que son particularmente sensibles o que están profundamente relacionadas con las variables fisicoquímicas que determinan el estado trófico de los cuerpos de agua, como lo son los nutrientes, la cantidad de clorofila a y la transparencia. A través de estos se han podido utilizar diferentes especies del zooplancton como indicadoras de los estados tróficos como oligotrofia, mesotrofia y eutrofia, e inclusive se han desarrollado diferentes índices, tanto usando como base la presencia de las especies como con sus abundancias.

Los estudios realizados para determinar el potencial ecológico usando al zooplancton como indicador son menores que aquellos para determinar el estado trófico, sin embargo, estos representan una gran base para indicar su eficacia como indicadores. Por otro lado, la DMA, aunque haya dejado fuera al zooplancton en primera instancia, no impide que posteriormente pueda ser incluido como otro EBC. Por lo cual, ha sido importante profundizar en su estudio y presentar si es posible las pautas necesarias para su incorporación. Bajo ese precepto en la presente tesis se busca determinar cómo el zooplancton puede ser un elemento útil para determinar la calidad del medio acuático, especialmente el potencial ecológico utilizando diferentes modelos y análisis con diferentes acercamientos, como a nivel de especies, abundancias, biomasas, proporciones y aprendizaje automatizado. Esto con los datos de las comunidades del zooplancton obtenidas a lo largo de una década dentro de un gran número de embalses localizados en la cuenca hidrográfica del Ebro, España.

Evaluación de la calidad del agua usando al zooplancton, una mini revisión

Durante los últimos 20 años, los trabajos que emplean al zooplancton para la determinación de la calidad del agua han ido en aumento, esto se debe principalmente al reconocimiento que se le ha dado a este grupo y su sensibilidad a los cambios en el ambiente. Este aumento de trabajos ha traído consigo la creación de varios índices utilizados en diferentes sistemas acuáticos. Para evaluar el estado trófico de diferentes cuerpos de agua se puede encontrar una variada cantidad de trabajos en diferentes partes del mundo, utilizando un grupo como modelo, por ejemplo, solamente rotíferos, cladóceros, copépodos o todas estas comunidades al unísono. Sin embargo, los estudios enfocados en determinar el estado ecológico dentro de la DMA usando al zooplancton, siguen siendo escasos en comparación.

Algunos estudios que utilizan a estos grupos del zooplancton tienen en común que han sido elaborados para uno o pocos cuerpos de agua. Como el caso del lago Erie en Estados Unidos, donde se probaron algunas métricas tanto del fitoplancton como del zooplancton para evaluar el estado trófico del lago. Del otro lado del océano Atlántico, en Reino Unido, encontramos el lago Loch Lomond, donde utilizando solamente al grupo de los rotíferos se determinó que las abundancias de las especies variaban a lo largo del gradiente trófico. Otros estudios, aunque solo hayan sido dentro un cuerpo de agua, poseen un registro de datos a lo largo del tiempo, como lo pueden ser años o décadas. Este es el caso de un estudio realizado en la parte central de Estonia, donde se obtuvieron las especies indicadoras tanto de eutrofia como de oligotrofia. Por otro lado, en la parte sur de Brasil se desarrolló un índice utilizando especies de los tres grupos del zooplancton. La elaboración de este índice fue para embalses y la determinación de su estado trófico y futuras predicciones. Sin embargo, en este trabajo solo se utilizaron muestras de siete embalses diferentes. Todos estos estudios previos, elaborados en un cuerpo de agua como aquellos desarrollados en pocos, siguen teniendo la limitación de no poseer una mayor representación de diferentes ambientes y comunidades, por lo que solo podrían ser aplicados al mismo cuerpo de agua o región donde fueron obtenidos los datos.

Dentro de los estudios en los cuales se utilizaron una mayor cantidad de cuerpos de agua, encontramos el índice de zooplancton en humedales (Wetland Zooplankton Index - WZI). Este estudio tomó como referencia 70 diferentes lagos en la zona de los grandes lagos, los cuales pertenecen una gran cuenca ubicada entre Canadá y Estados Unidos. Aunque este índice obtuvo una cantidad significativa de especies indicadoras de diferentes niveles de calidad de agua, los propios autores mencionan que este índice solo podría ser usado en la región donde los lagos se ubicaban y que otros métodos, métricas y enfoques deberían ser probados, para no tener solamente la limitante de un acercamiento a nivel de especies.

La mayoría de los estudios anteriores fueron basados solamente en la presencia o absencia de ciertas especies, pero ¿qué hay acerca de sus abundancias? Uno de los estudios más potentes que se tienen registrados usando las abundancias de los grupos del zooplancton, fue elaborado para lagos de Polonia. En este estudio se evaluaron más de 70 lagos y se obtuvieron las diferentes fórmulas y métricas para poder determinar el estado trófico a través de rotíferos y microcrustáceos. Posteriormente utilizando los indicies obtenidos por las fórmulas anteriores, se realizó un estudio en 16 lagos mediterráneos ubicados en la región de Grecia. Los estudios detectaron que las abundancias de las comunidades zooplanctónicas fueron útiles para discriminar y determinar el estado trófico de los lagos. Sin embargo, debido a los objetivos de estos y de los estudios anteriormente mencionados, estos no evaluaron a las comunidades para determinar el potencial ecológico, dejando así un vacío de información que podría usarse para la inclusión del zooplancton dentro de la DMA.

Dentro de los pocos trabajos evaluando el potencial ecológico dentro de cuerpos de agua dulce podemos encontrar dos estudios recientes. El primero llevado a cabo en Polonia, presentando el índice de zooplancton para lagos polacos (Zooplankton Index for Polish Lakes' Assessment - $ZIPLA_S$). Este índice fue creado a través de 45 lagos diferentes utilizando diferentes métricas enfocadas a especies, proporciones y abundancias. Con base al índice, se pudieron identificar diferentes especies asociadas a los diferentes niveles tróficos, así como se proponen los límites o rangos en el que se encuentran las clases del potencial ecológico. En comparación con el resto de los trabajos, el índice es bastante completo, pudiendo mejorar en pocas áreas, como la implementación de grupos funcionales y aprendizaje automatizado. En el segundo estudio, localizado en el norte de Portugal, se utilizó un enfoque a nivel de especies y grupos funcionales. Aunque este estudio se realizó en diferentes épocas del año, tiene la limitante de que solamente se contó con cuatro embalses diferentes, los cuales se encontraban relativamente cerca unos de los otros. Además, estos embalses no tenían representación de sistemas con baja calidad de agua, ya que la mayor parte del tiempo fueron clasificados con buena calidad del agua. No obstante, estos estudios indican que los grupos del zooplancton podrían ser un buen Elemento de Calidad Biológica dentro de la DMA.

Al igual que el estudio anterior, localizados dentro de la península ibérica, se realizó otro estudio tanto para estado trófico como potencial ecológico utilizando a las comunidades zooplanctónicas, pero esta vez en España. Este estudio estuvo enfocado en la cuenca del Júcar y conto con datos de 20 embalses durante dos épocas distintas. Este trabajo logro identificar diferentes especies como indicadoras de baja calidad de agua, pero no especies indicadoras de buena calidad, lo cual podría dificultar a futuro una implementación de un índice o protocolo para determinación de ambientes oligotróficos.

Para un caso más específico y centrado en la misma área de trabajo que el presente estudio, la cual es la cuenca del Ebro, tenemos el Índice trófico de embalses de zooplancton (Zooplankton Reservoir Trophic Index - ZRTI). El cual fue diseñado por la confederación a cargo de la cuenca del Ebro. Aunque este índice evalúa a las especies del zooplancton para la determinación del potencial ecológico de los embalses usando una aproximación a través de análisis multivariante. Su aplicación se ve un poco limitada al solamente evaluar la posición en donde fueron colocadas las especies dentro de un análisis ACC y sin tomar en cuenta otros rasgos, como el de las abundancias y grupos funcionales. El acercamiento que tuvo este trabajo y el estudio anterior se podrían considerar como un punto de referencia para la presente tesis y se verá más a fondo en el capítulo dos.

En general podemos apreciar que estudios que se han realizado anteriormente poseen información de uno o pocos sistemas para la elaboración de los índices. Son pocos los estudios en los que la cantidad de información recopilada de lagos o embalses son mayores a 10. Esto se debe principalmente por la falta de presupuesto en los proyectos. Por lo cual, aquellos programas de monitoreo de varios años y del que se obtienen muestras de diferentes cuerpos de agua son vitales para conseguir una mayor y mejor cantidad de información de los sistemas acuáticos a través del tiempo. El tener una mayor cantidad de datos abre las puertas a realizar y ejecutar un mejor y más robusto análisis de datos, para así poder tener mejores resultados y la creación de índices más fiables.

Por lo tanto, un punto fuerte de esta tesis es la rica base de datos que se fue obteniendo a lo largo del tiempo durante todo el proyecto. A diferencia de otros estudios tiene representaciones de embalses clasificados en diversas categorías, tanto del estado trófico como del potencial ecológico. Esto nos da un amplio espectro tanto de las variables fisicoquímicas de los embalses como de las especies presentes en ellos y como varían entre cada sistema, lo cual podrá ser beneficioso para determinar aquellas especies y/o grupos que puedan ser buenas indicadoras.

Objetivos de la tesis

La presente tesis tiene como objetivo principal:

- Evaluar si las comunidades zooplanctónicas son buenos indicadores para la determinación de la calidad del agua en los embalses del Ebro. Esto, a través de una aproximación a nivel de especies, abundancias, grupos funcionales y aprendizaje automático.

Con el objetivo anterior establecido, los objetivos específicos de la tesis son:

1) Analizar los cambios en la composición de especies en seis embalses durante dos temporadas distintas (verano – otoño) y determinar a qué factores ambientales se encuentran asociadas.

2) Describir las especies de los tres principales grupos de zooplancton (rotíferos, cladóceros y copépodos) que pueden ser utilizados como indicadores de los diferentes estados tróficos y potencial ecológico en los embalses situados en la cuenca del Ebro.

3) Establecer si las abundancias y biomasas del zooplancton pueden ser utilizadas para diferenciar entre los diferentes niveles del estado trófico y el potencial ecológico de los embalses.

4) Determinar la calidad de agua a través de la utilización de grupos funcionales del zooplancton y de modelos predictivos usando el aprendizaje automático.

Metodología

La metodología para la obtención de los datos y resultados de la presente tesis fue organizada en diferentes secciones. La sección principal, en ella se establece la metodología que se siguió durante los años en los que se realizaron las salidas a campo y muestreo de los embalses. También, se estableció la forma adecuada para la obtención de las variables ambientales durante los muestreos de cada embalse. Estas variables fueron conformadas por los parámetros fisicoquímicos de la columna de agua y los datos morfológicos específicos de cada embalse. Además de la correcta forma de recolección, fijación y almacenamiento de las muestras biológicas, que fueron conformadas por el fitoplancton y el zooplancton. Aunque los objetivos de esta tesis no engloban al fitoplancton, sus comunidades fueron usadas como variables para el análisis del zooplancton. Por otra parte, se especifica la forma en la que se identificaron las especies del zooplancton, así como la forma en que se determinaron tanto el potencial ecológico y estado trófico de cada embalse.

En los diferentes capítulos de la tesis, se establecen la metodología seguida para el análisis de datos con base en cada uno de los objetivos propuestos. Una vez que se obtuvieron todos los datos de campo, se empezaron a elaborar las matrices de datos correspondientes. Estas matrices fueron las siguientes: i) datos ambientales, las cuales fueron compuestas por las variables fisicoquímicas y morfológicas de los embales. ii) listado de especies zooplanctónicas presentes en los embalses con sus respectivas abundancias y biomasas. iii) listado de especies pertenecientes al fitoplancton con sus abundancias y biomasas.

Metodología general

El rio Ebro posee una cuenca hidrográfica de 86.000 km^2, lo que lo convierte en la mayor cuenca de España y atraviesa siete comunidades autónomas: Cantabria, Castilla y León, La Rioja, País Vasco, Navarra, Aragón y Cataluña. Por su gran extensión, también lo coloca como una de las mayores cuencas hidrográficas de toda el área mediterránea. Los datos de la presente investigación se obtuvieron a través de 304 eventos de muestreo o muestras obtenidas de 66 diferentes embalses ubicados a lo largo de la cuenca. Las campañas de muestreo se realizaron durante la época de verano de los años 2010 a 2019. Dentro de cada embalse, se estableció un punto de muestreo en la parte más profunda, el cual estuvo localizado aproximadamente a 300-500 metros de la pared de la presa. En este punto se midieron las variables fisicoquímicas y se tomaron muestras de fitoplancton y zooplancton. La estimación del estado trófico y potencial ecológico de los embalses fue por medio del índice de Estado Trófico y de la metodología traspuesta por la legislación española y la directiva marco del agua.

Las muestras de zooplancton se recogieron utilizando una botella Ruttner vertical con capacidad de 2.7 litros. En cada embalse se tomaron dos botellas Ruttner para obtener un total de 5.4 litros de muestra de agua, posteriormente se filtró a través de un filtro Nytal de 30 µm de apertura de luz. De la misma forma se tomaron muestras de fitoplancton, que, aunque no fueron analizadas en la presente tesis, los datos obtenidos de esta comunidad si fueron utilizados para los análisis posteriores. La profundidad a la que se recogieron las muestras se estableció al inicio de la oxiclina en cada uno de los embalses. Las muestras obtenidas se fijaron con lugol y se almacenaron dentro de una nevera fría en frascos de vidrio herméticos. Las especies de zooplancton posteriormente se identificaron en el laboratorio usando literatura especializada y los conteos para determinar la cantidad de especies presentes y las abundancias de estas fueron realizados bajo microscopio invertido.

Metodología por capítulos

En el **capítulo 1**, se abordó con un enfoque de ecología de las comunidades zooplanctónicas y se examinó si había diferencias entre ellas dentro de seis embalses en dos diferentes épocas (verano y otoño). Para esto se utilizaron índices de diversidad, análisis de similitud (ANOSIM) y porcentaje de similitud (SIMPER). Se realizaron también análisis multivariantes como el Análisis de Correspondencias Canónicas (ACC) con dos diferentes enfoques. El primero para determinar la relación de las variables ambientales con las especies presentes y el segundo, entre las variables ambientales y los grandes grupos del zooplancton (rotíferos, cladóceros y copépodos).

Una vez teniendo una idea general de los cambios de las comunidades del zooplancton en embalses de diferente calidad de agua, se procedió a determinar las especies que podrían ser indicadoras del potencial ecológico y del estado trófico. Así que en el **capítulo 2**, se crearon matrices de variables ambientales y de zooplancton con los datos de las 304 ocasiones de muestreo que se obtuvieron. Para determinar las especies que más contribuyeron a los cambios dentro de las comunidades se realizó un análisis de porcentaje de similitud. Así mismo, para determinar la relación entre las variables ambientales y las especies de zooplancton, realizamos un análisis de correspondencias canónicas. Este mismo análisis se usó para determinar cuáles especies estaban más relacionadas con las variables que determinan la calidad del agua. Por último, para obtener las especies indicadoras de los diferentes estos tróficos y potencial ecológico se realizó un análisis de Valor Indicador (IndVal), esto con la ayuda del lenguaje de programación R.

Después de obtener las especies indicadoras, en el **capítulo 3** propusimos una serie de métricas con base a las abundancias en individuos por litro (ind L^{-1}) y biomasas en peso seco (PS) del zooplancton para evaluar la calidad de agua. Las métricas propuestas fueron: a) ZOO (zooplancton total), b) LZOO (zooplancton de gran tamaño), c) SZOO (zooplancton de pequeño tamaño), d) ZOO:CHLA (proporción entre la clorofila a y el zooplancton), e) ZOO:PHYTO (proporción entre el fitoplancton y el zooplancton), f) Principales grupos de zooplancton (rotíferos, copépodos y cladóceros), g) Géneros u ordenes seleccionados (daphnidos, copépodos ciclopoideos y calanoideos). Para calcular las relaciones entre las métricas y las variables ambientales se emplearon correlaciones de Pearson y regresiones múltiples. Con la finalidad de probar si las métricas propuestas eran validas y verificar si había diferencias significativas entre los diferentes niveles del estado trófico y potencial ecológico se utilizó la prueba de t de Student.

Finalmente, en el **capítulo 4**, se utilizó un enfoque a nivel de grupos funcionales y aprendizaje automático. Primero, para obtener los grupos funcionales del zooplancton, se utilizaron diferentes rasgos funcionales: peso corporal de los adultos, hábitat, grupo trófico, forma de reproducción y tipo de alimentación. Con la información anterior se creó una matriz con datos numéricos y categóricos de las especies y sus correspondientes rasgos funcionales. Posteriormente se creó una matriz de disimilitud y para asignar las especies en grupos, se aplicó a esta matriz un análisis de agrupación jerárquica utilizando el método de Ward. En este capítulo también se utilizaron las matrices tanto de variables ambientales como del fitoplancton. Una vez teniendo las matrices, se procedió a diferentes análisis estadísticos y multivariantes tales como; correlaciones de Pearson, análisis de componentes principales y análisis de redundancia.

Para la determinación del estado trófico y del potencial ecológico mediante el aprendizaje automático se utilizaron las matrices de los grupos funcionales y las variables fisicoquímicas de los embalses. Como modelo de aprendizaje automático para predecir la calidad del agua se eligió el método de bosques aleatorios. Así, realizamos dos diferentes análisis de bosques aleatorios para determinar y establecer qué grupos funcionales eran los más importantes para determinar el estado trófico y el potencial ecológico. Después, se crearon modelos para predecir la calidad del agua con los datos de los grupos funcionales, estos modelos se realizaron dividiendo los datos en un conjunto de datos de entrenamiento (80% de los datos seleccionados aleatoriamente) y los datos de validación (20% de los datos). Y finalmente, para conocer y establecer los límites entre cada categoría tanto del estado trófico como del potencial ecológico, utilizamos árboles de clasificación y regresión con los datos de las abundancias de los grupos funcionales más importantes.

Resultados y discusión

La presente tesis fue creada como una investigación para determinar si el zooplancton puede ser usado como indicador de la calidad de agua. Este acercamiento se debe a que las comunidades de este grupo fueron excluidas como Elementos de Calidad Biológica, dentro La Directiva Marco Europea del Agua. Con esta exclusión se levantó una gran polémica, dado que el fitoplancton fue el único grupo planctónico que si fue incluido. Así que, por lo tanto, se evaluó a los grupos del zooplancton como herramientas para la determinación de la calidad del agua con la ayuda del conjunto de datos obtenidos a través del gran número de muestras de los embalses con diferente estado trófico y potencial ecológico muestreados a lo largo de toda la cuenca del Ebro.

Los embalses del Ebro, estado trófico, potencial ecológico y sus variables ambientales

En primer lugar, después de diez años de muestreos, se obtuvieron 304 muestras de todos los embalses, cada una de estas muestras fueron compuestas de las variables ambientales antes establecidas, así como los datos de los conteos de los grupos zooplanctónicos. Para establecer la diferencia entre cada una de estas muestras, fueron nombradas con base al embalse y al año muestreado, por ejemplo, Llauset 2018. Por razones logísticas y de presupuesto, no todos los embalses fueron muestreados cada año, sin embargo, muestreamos cada embalse dentro de un rango de tres a cuatro años como mínimo. En comparación con otros estudios, el número de embalses muestreados es bastante grande, ya que varios artículos han usado como modelo un solo cuerpo de agua, otros tantos menos de una docena, mientras que son muy pocos los que sobrepasan los cincuenta cuerpos de agua diferentes.

Debido a la morfología que presenta toda la cuenca del Ebro y por los diferentes usos para los que fueron construidos los embalses, nos encontramos con una gran diversidad de tamaños y profundidades de estos. Siendo de los más pequeños aquellos que no superaban un hectómetro de volumen, mientras que los más grandes llegaron a tener más de mil hectómetros de volumen. En el caso de la profundidad nos encontramos también un gran rango, ya que los menos profundos rondaron entre los dos metros, por otro lado, los más profundos tuvieron entre 70 a 120 metros. Por esta misma razón, también se encontraron rangos muy amplios en las diferentes variables fisicoquímicas dentro de los embalses. Dentro de todos los parámetros medidos, los que tuvieron una mayor importancia para los diferentes análisis de esta tesis fueron los siguientes. La concentración de clorofila a, que vario de los 0.4 µg/L hasta 51.9 µg L^{-1} La cantidad de fosforo total, entre los 0.65 µg L^{-1} y 186 µg L^{-1}. La profundidad hasta la que se pudo apreciar el disco de Secchi, yendo desde los 0.23 metros hasta los 18 metros.

El listado lo completan la temperatura superficial del agua con rangos de entre 10.3°C y 28.1°C y el oxígeno disuelto que vario de los 2.5 mg L^{-1} hasta 14.38 mg L^{-1}.

De los parámetros anteriores, los tres primeros (clorofila a, fosforo total y la profundidad del disco de Secchi) son los más usados para determinar el estado trófico de los cuerpos de agua y con base a estos se calculó el índice de estado trófico. Este índice nos indica que la gran mayoría de los embalses fueron catalogados como oligotróficos o mesotróficos, ya que cada una de estas categorías tuvo 123 muestras. Mientras que aquellos embalses clasificados como eutróficos o hipereutroficos fueron 55 y 3 respectivamente. Por otro lado, siguiendo las pautas que marca la DMA para determinar el potencial ecológico con sus correspondientes categorías, usando también parámetros como la comunidad fitoplanctonica y la cantidad de oxígeno disuelto en el agua. Se obtuvo que 99 embalses se catalogaron como bueno o superior, mientras que la gran mayoría se encontraron en la categoría de moderado, siendo 202 embalses. Finalmente, muy pocos embalses fueron catalogados en los niveles bajos, teniendo solo 3 como pobre y ninguno clasificado como malo.

Artículos publicados: El zooplancton como bioindicador

El **primer capítulo** de esta tesis fue empleado como un análisis exploratorio para verificar como estaban conformadas las comunidades zooplanctónicas en solamente seis embalses durante las temporadas de verano y otoño, así como su relación con las variables ambientales. Se contabilizaron entre todos los embalses 40 especies diferentes pertenecientes a los tres grandes grupos, así como también el mejillón cebra *Dreissena polymorpha.* Aunque a lo largo de los años y con diferentes grupos de investigación se habían descrito las especies del zooplancton en una gran cantidad de embalses a lo largo de España. En este capítulo se presentan por primera vez las especies en el embalse Ullibarri-Gamboa, esto en conjunto a las especies registradas en los otros embalses nos indica algunas cosas. Primero, que existen aún muchos sistemas que no se han descrito las especies presentes. Segundo, los listados taxonómicos y registros que se tienen están desactualizados. Tercero, que la diversidad planctónica puede ser aún más grande de lo que se tiene registrado y pensado.

Bajo un acercamiento a nivel de grupos del zooplancton con el ACC, pudimos detectar que los microcrustáceos estaban fuertemente relacionados con las variables que determinan el estado trófico, esta asociación se había reportado previamente para otros embalses en la cuenca. Por ejemplo, los cladóceros estaban más relacionados con la cantidad de clorofila a, siendo bastante sensibles a los cambios que se producen en las comunidades fitoplanctónicas. Mientras que los copépodos estuvieron relacionados con la transparencia del disco de Secchi, esto probablemente por la composición de las especies de copépodos que los habitan, siendo algunos de estos los calanoideos como *Copidodiaptomus numidicus* y *Eudiaptomus vulgaris*, mientras que un ciclopoideo con gran presencia fue *Acanthocyclops americanus*. Hablando de especies de copépodos, en el caso de *Neolovenula alluaudi* se tenía registrada la presencia en uno de los embalses desde décadas atrás, sin embargo, durante el presente estudio no se detectó. Esto puede deberse a múltiples motivos, tales como la depredación por peces, el haber sido desplazada por otra especie exótica, o que las condiciones ambientales hayan cambiado y afectado sus poblaciones. Por lo tanto, para conocer los cambios en las comunidades es recomendable la constante monitorización de los cuerpos de agua.

Un punto para destacar del presente capitulo, es el hecho que se detectó por primera vez la presencia del mejillón cebra dentro del embalse de La Sotonera, el cual se creía libre de este invasor. Esta especie exótica, originaria de la región entre los mares Negro, Caspio y Aral, se ha extendido por gran parte del mundo, y ha ido desplazando a las especies nativas. Actualmente se encuentra clasificado como una de las especies exóticas más dañinas del mundo. Dentro de la cuenca del Ebro, como en muchas otras regiones, no solamente causa problemas ecológicos, sino que también se ha reportado que ocasiona pérdidas económicas. Ya que se han encontrado grandes poblaciones del mejillón dentro de las diferentes estructuras en los embalses y represas, haciendo que se tapen por el exceso de animales, provocando un gran gasto en tiempo y dinero para su remoción, aunado a los trabajos de mantenimiento. Se cree que el mayor medio de propagación dentro de los embalses del Ebro de este mejillón es a través de los mismos humanos. Principalmente por pescadores y personas que navegan en los embalses sin una correcta desinfección de los botes y equipamiento que ha estado en contacto con el agua, facilitando de esta forma que se propaguen las larvas.

Los datos obtenidos del primer artículo nos dieron una idea de la presencia de las especies del zooplancton presentes en la región y como estas se relacionaban con las variables ambientales, esto sirvió para desarrollar y plantear de una mejor forma el resto de los capítulos de la tesis. Además, de que nos sugiere que el conocimiento de las especies de zooplancton y el cambio en su composición a través del tiempo pueden ser una herramienta para el manejo de embalses y cuencas. Por ejemplo, detectando oportunamente especies invasivas para aplicar las medidas necesarias para su control y evitar su propagación o en caso de que fuera oportuno, su remoción.

Una vez teniendo una idea general de como las comunidades estaban compuestas dentro de pocos embalses y los parámetros que tenían mayor influencia en ellas. Se creo una base de datos con todos los datos disponibles de años anteriores y de muestreos que se fueron realizando, siendo un total de 304 muestras. La riqueza de especies obtenidas durante todo el periodo de estudio fue un total de 169 especies de zooplancton. La mayor cantidad de especies pertenecieron a los rotíferos, con un total de 115 especies pertenecientes a 36 géneros diferentes, seguido de los cladóceros con 36 especies distribuidas en 15 géneros. Mientras que los copépodos estuvieron conformados por 17 especies en 11 géneros. Los géneros que tuvieron una mayor representación de especies fueron *Brachionus, Lecane Polyarthra, Synchaeta* y *Trichocerca* en los rotíferos. En el caso de los cladóceros y copépodos fueron *Daphnia* y *Cyclops* respectivamente.

En diferentes estudios efectuados en diversas regiones alrededor del mundo se han usado a las especies de varios grupos, por ejemplo, peces, plantas acuáticas y fitoplancton como indicadoras de la calidad de agua. Aunque han sido establecidas diferentes metodologías para cada grupo, generalmente se ha utilizado la presencia de especies asociadas una buena o mala calidad de agua. Usando un enfoque similar, fue desarrollado el **segundo capítulo** de la presente tesis. En el cual utilizando diferentes análisis reportamos que 14 especies pueden ser usadas como indicadoras para el estado trófico. Mientras que este número se reduce a solo seis para el potencial ecológico, esto nos sugiere que las especies del zooplancton son más sensibles para diferenciar entre los niveles del estado trófico.

Varios trabajos han reportado que los copépodos ciclopoideos y que algunas especies pertenecientes al género *Brachionus* en conjunto con la especie *Keratella cochlearis* en su forma tecta son buenas indicadoras de eutrofia. Esto está en concordancia con los resultados que obtuvimos a través de los diferentes análisis, ya que *K. cochlearis* tecta y el ciclopido *A. americanus* fueron unas de las especies indicadoras de embalses eutróficos y de potencial ecológico pobre utilizando el método de IndVal. Mientras que en el ACC estas especies estuvieron fuertemente relacionadas con las variables clorofila a, fosforo total y turbidez. Generalmente cuando estas variables se encuentran en altas cantidades en algún cuerpo de agua, indican eutrofia o un bajo potencial ecológico. Tanto *Collotheca pelagica* como *Brachionus angularis, Pompholyx sulcata y Daphnia cucullata* fueron otras de las especies indicadoras de baja calidad de agua.

En caso contrario, usando el método de IndVal, la cantidad de especies que indicaran oligotrofia o un potencial ecológico bueno o superior solo estuvo compuesto por el cladocero *Daphnia longispina*. A pesar de que solo una especie fuese indicadora, se considera un buen avance, ya que previamente otros estudios bajo un enfoque similar no habían podido establecer alguna especie indicadora para condiciones oligotróficas. También detectamos que las especies de pequeño tamaño fueron las responsables principalmente en el cambio en la composición de especies, esto contrastando el nivel más alto y bajo tanto del estado trófico como del potencial ecológico.

La utilización de especies indicadoras con organismos planctónicos para la estimación del potencial ecológico dentro de la DMA actualmente solo es llevada a cabo con el fitoplancton. Sin embargo, con los datos de esta investigación, sugerimos que las especies obtenidas podrían ser buenos indicadores para los embalses en la cuenca del Ebro. Por otro lado, estas especies deberían probarse en otras regiones y cuerpos de agua como lagos para comprobar su eficacia para que posteriormente, el zooplancton pueda ser incluido dentro de la DMA.

Una diferencia que se puede notar dentro de los sistemas acuáticos con diferentes niveles de eutrofia es la cantidad de organismos presentes en ellos. Esta abundancia o densidad está condicionada por la cantidad de recursos disponibles para mantener a las poblaciones. Mientras que los cuerpos de agua oligotróficos contienen baja cantidad de fosforo, el cual es el nutrimento limitante para el fitoplancton, este hace que no aumente su abundancia. En los eutróficos este recurso es mucho más abundante provocando que las algas incrementen su densidad. Al haber más disponibilidad de microalgas el zooplancton poco tiempo después también aumenta sus abundancias. Entonces, ¿podríamos determinar el nivel de eutrofia de los embalses usando como base la abundancia del zooplancton presente en ellos? Bajo esta interrogante fue elaborado el **tercer capítulo** de la presente tesis.

Para investigar esto no solo utilizamos las abundancias del zooplancton, sino que también calculamos la biomasa de cada especie presente en los embalses. Al igual que en el capítulo anterior, debido a la gran diversidad tanto en las variables morfométricas como ambientales que presentaron los embalses, las diferencias entre máximos y mínimos fue muy amplia. Para el caso del primero, la menor abundancia del zooplancton total fue tan solo 6.76 ind L^{-1} , mientras que el máximo ascendió hasta 2758 ind L^{-1}, mientras que la biomasa tuvo mínimos y de máximos de 0.45 µg PS y 1971 µg PS respectivamente.

Se analizó la comunidad zooplanctónicas en conjunto, así como por grupos, esto a través de las diferentes métricas planteadas previamente. De manera general se pudo notar que la mayoría de las métricas tenían una tendencia positiva al aumentar sus números conforme aumentaba el nivel de eutrofia, esto es fue más evidente con el estado trófico que con el potencial ecológico. Las métricas que no mostraron una tendencia positiva tan marcada fueron SZOO y ZOO:PHYTO, mientras que ZOO:CHLA tuvo una tendencia negativa, esto debido que al aumentar la cantidad de zooplancton disminuía la proporción con la cantidad total de clorofila a.

Para poder establecer la categoría en la que se encuentra un cuerpo de agua, la DMA utiliza varias métricas como se ha dicho anteriormente, sin embargo, a esta directiva le es de gran interés poder diferenciar los embalses que se encuentran en un buen potencial ecológico de los demás (moderado, pobre y malo). Así que, pusimos a prueba si nuestras métricas pudieran ser útiles para diferenciar entre esos niveles, pero también para diferenciar entre las diferentes categorías del estado trófico. Los resultados de la prueba de *t* indico que la gran mayoría de las métricas propuestas tuvieron diferencias significativas para diferenciar entre bueno o superior del resto. Mientras tanto, para diferenciar los diferentes niveles de estado trófico, las métricas fueron variando, siendo algunas más sensibles que otras. De igual forma, empleamos la misma prueba para diferenciar la oligotrofia de la eutrofia, teniendo resultados similares a los obtenidos del potencial ecológico, siendo que una gran cantidad de métricas fueron significativas para diferenciarlos.

Algo que tomar en cuenta usando las abundancias y las biomasas, es que, aunque el segundo depende del primero, hay que tener en cuenta que grupos son los que están presentes. Ya que, si dentro de una comunidad la gran mayoría fueran rotíferos, la biomasa total no sería tan grande, sin embargo, si la mayor parte de esa comunidad fuese conformada por cladóceros de gran tamaño y copépodos, la biomasa seria considerablemente más alta. Este factor puede hacer que la biomasa entonces pueda ser más o menos sensible en diferenciar la calidad del agua. Esto lo podemos apreciar con algunas métricas, por ejemplo, la biomasa total del zooplancton que fue capaz de separar los ambientes oligotróficos de los mesotróficos, mientras que la abundancia total no lo pudo hacer. Casos parecidos los tenemos otras métricas, que dependiendo si se usó abundancias o biomasas fueron capaces o no de diferenciar entre los niveles de estado trófico o potencial ecológico.

Los resultados obtenidos en este capítulo son similares a otros estudios que utilizaron las abundancias del zooplancton por grupos funcionales o por órdenes taxonómicos dentro de la región del mediterráneo. Por lo tanto, las métricas propuestas que emplean densidades y biomasas del zooplancton podrían ser utilizadas como herramientas dentro de la DMA para poder conseguir una correcta clasificación de los embalses.

En comparación con el resto de los estudios dentro del área de ecología acuática, pocos son los trabajos que han tenido un enfoque con base a los grupos funcionales del zooplancton. Dentro de los estudios realizados con estos grupos funcionales, aun son más escasos los estudios usados con la finalidad para determinar el estado trófico de algún cuerpo de agua. Así que, por lo tanto, en el **último capítulo** de esta tesis se determinaron los grupos funcionales presentes en los embalses, y si estos podrían ser usados para determinar el estado trófico y el potencial ecológico. Aunado a que también se implementó un acercamiento novedoso para esta área, el del aprendizaje automático.

De toda la comunidad zooplanctónica presente en los embalses del Ebro, se lograron identificar cinco grandes grupos funcionales, estos fueron copépodos filtradores, copépodos raptoriales, cladóceros, rotíferos microfagos y rotíferos raptoriales. En general, los grupos funcionales aumentaron sus densidades mientras aumentaba el estado trófico, con excepción de los rotíferos raptoriales. Por otro lado, los valores ambientales que tuvieron más importancia dentro de la cuenca fueron la comunidad fitoplanctónica, y las variables morfológicas como el volumen de agua y el porcentaje de llenado que se encontraba cada embalse. Otras variables como la clorofila a y el fosforo total estuvieron relacionadas con los copepodos raptoriales, compuestos por los ciclopoideos, mientras que a los copepodos filtradores y cladóceros fueron más relacionados a la temperatura, amonio, conductividad y alcalinidad.

A lo largo de diversos cuerpos de agua se ha notado que los copepodos raptoriales se han encontrado en grandes cantidades en sistemas eutróficos, e inclusive sin ser tan afectados por florecimientos cianobacteriales. También los integrantes de este grupo han sido fuertemente correlacionados con la cantidad de fosforo total, siendo considerados como indicadores de eutrofia, lo cual está en concordancia con los resultados tanto de este capítulo, como el segundo y el tercero. Por otro lado, observamos que, en aquellos embalses con buena calidad de agua, hubo una mayor dominancia y baja abundancia de grupos filtradores, como lo son los calanoideos y los cladóceros, esto igual se ha reportado en diferentes estudios, donde en cuerpos de agua oligotróficos o tendientes a la oligotrofia son dominados por *Daphnia* y calanoideos. Por lo cual, señalamos que la presencia y baja densidad de estos se podría usar como indicador de ambientes poco productivos.

Con el paso del tiempo nuevas métodos y tecnologías han ayudado al entendimiento del medio natural, esto ha ido en aumento especialmente en las últimas dos décadas. El aprendizaje automático es una nueva forma de poder entender la información que se tiene a través de modelos que pueden ser sencillos hasta robustos. Todos estos modelos pueden ser utilizados para diferentes fines, incluyendo datos ecológicos. Recientemente se ha evaluado, dentro de las diferentes técnicas que abarca el aprendizaje automático, cuáles han sido las más eficientes dentro de diferentes sistemas naturales. En el caso de los sistemas acuáticos, se encontró que la técnica de bosques aleatorios fue la que obtuvo un mayor rendimiento. Por lo cual, en nuestro estudio, a través de la técnica de bosques aleatorios, determinamos cuales fueron los grupos funcionales del zooplancton que tuvieron más peso al determinar el estado trófico y el potencial ecológico. Teniendo como resultado que, para ambos, los copepodos ciclopoideos fueron los que tuvieron una mayor importancia, seguido de los calanoideos y cladóceros.

Los rotíferos fueron los que presentaron una menor importancia al igual que los resultados obtenidos a través de los grupos funcionales, ya que no fueron tan sensibles como los demás grupos. Esto también se pudo ver en el capítulo anterior, ya que las métricas establecidas con los rotíferos fueron de las menos sensibles para la diferenciación entre los niveles de estado trófico y potencial ecológico. En contra parte, algunas especies de rotíferos si fueron indicadoras de eutrofia en el segundo capítulo, y en otros estudios se han mencionados que los rotíferos podrían ser también utilizados como buenos indicadores del estado trófico. Sin embargo, en el caso del presente estudio los datos nos sugieren que los microcrustáceos serian mejores elementos para la evaluación de los embalses.

La precisión de los diferentes modelos predictivos vario en función de los grupos usados y el elemento a predecir, esta variación fue entre el 44-77%, siendo que obtuvieron los mejores desempeños en aquellos modelos que usaron a los ciclopoideos, seguidos de los cladóceros y los microcrustaceos en general para predecir el estado trófico. Mientras que, para predecir el potencial ecológico, los modelos más eficientes fueron aquellos que emplearon los datos de los calanoideos seguido de los microcrustaceos. La precisión dentro de los modelos que abarcan datos ecológicos suele ser en general más bajos que contra modelos predictivos de otro tipo, ya que, en la ecología, hay muchos factores o variables que no se pueden controlar y que podrían ejercer algún tipo de sesgo. Sin embargo, el poder conseguir modelos con una alta precisión, puede representar un buen avance para un mejor y correcto manejo de los recursos hídricos, como lo son lagos, embalses y ríos.

Un argumento en contra de la inclusión del zooplancton como un elemento biológico de calidad, es que no se encuentra establecido los límites en términos de abundancias o biomasas para diferenciar entre los diferentes niveles del potencial ecológico. Nosotros pudimos abordar este problema utilizando las abundancias del zooplancton, y no solamente para el potencial ecológico, sino que también para el estado trófico. Utilizando los árboles de clasificación y regresión pudimos fijar los límites del potencial ecológico para diferenciar el estado bueno o superior del moderado fueron: calanoides <1.4 ind L^{-1}, ciclopoides <0.38 ind L^{-1} y cladóceros <19.36 ind L^{-1}. Mientras que para el estado trófico entre condiciones oligotróficas y eutróficas fueron: calanoides <3.1 ind L^{-1}, ciclopoides <0.76 ind L^{-1} y cladóceros <19.04 ind L^{-1}.

Se observó que la separación de categorías entre ambas clasificaciones, con algunos grupos funcionales fueron similares. En el caso de los cladóceros que la abundancia para diferenciar entre oligotrofia de eutrofia y bueno de moderado fue mínima. Mientras que en el caso de los copépodos esa diferencia varía entre 1 y 2 ind L^{-1}, lo cual en términos de abundancia es un margen muy bajo. Por lo cual, se podría emplear ambas mediciones para la determinación tanto del potencial ecológico y estado trófico. Estos resultados pueden ser un buen punto de partida para la validación de estos límites en otros embalses localizados en otras regiones y el establecimiento de los mismos en otros sistemas acuáticos como lagos, lagunas y ríos.

Los resultados obtenidos durante la elaboración de la presente tesis indican que el zooplancton puede ser utilizado para la determinación tanto del estado trófico como del potencial ecológico en los embalses de la cuenca del Ebro. Esto a través de diferentes enfoques al analizar los datos de las comunidades zooplanctónicas, tanto de forma de especies indicadoras, abundancias y/o biomasas, grupos funcionales y aprendizaje automático.

Si la DMA no tiene contemplado a este grupo planctónico dentro de su programa ¿Por qué deberíamos tomar muestras de zooplancton? la obtención de las muestras del zooplancton no implicaría un mayor gasto dentro de los presupuestos en los muestreos de los cuerpos de agua, ya que tiene una metodología de muestreo bastante similar al fitoplancton. Durante las campañas de muestreo se pueden obtener muestras de zooplancton de forma paralela junto al fitoplancton utilizando solamente una red de apertura de luz más grande y con formas de preservación similares.

Así que, sugerimos que el zooplancton podría ser incluido dentro de la DMA como un elemento de calidad biológica. Si son empleados en conjunto con los datos que pueden ser obtenidos por el fitoplancton se podría tener una visión más grande y completa del ambiente, además de que ofrecerán una determinación más precisa de los cuerpos de agua.

Consideraciones finales y conclusiones

La presente tesis doctoral fue enfocada en el zooplancton como indicador de la calidad del agua, en específico para la determinación del estado trófico y el potencial ecológico. Este trabajo utiliza al zooplancton tanto a nivel de especies, abundancias, grupos funcionales y la implementación de aprendizaje automatizado. La metodología y resultados obtenidos de este trabajo también debería ser probada en otros sistemas acuáticos como lagos, esto con la finalidad de que posteriormente pueda ser incluido dentro de la DMA como un elemento biológico de calidad.

1. Dado que las variables ambientales que tuvieron una mayor influencia en la comunidad de zooplancton fueron la clorofila a, el fósforo total, la comunidad de fitoplancton, el volumen de agua y el tamaño del embalse, significa que el zooplancton posee sensibilidad a los cambios en el medio ambiente y puede ser un buen indicador de la calidad del agua. Por lo tanto, el zooplancton podría utilizarse para determinar el estado trófico y el potencial ecológico

2. Reportamos por primera vez las especies presentes en el embalse de Ullibarri-Gamboa. Estas especies fueron los cladóceros *B. longirostris, C. pulchella, D. cucullata, D. longispina*, el copépodo *A. americanus*, los rotíferos *A. fissa, B. angularis, C. unicornis, H. oxyuris, K. cochlearis, P. dolichoptera, P. major, S. pectinata* y el mejillón cebra *D. polymorpha*. Esto indica que todavía hay muchos cuerpos de agua en los que se desconoce la fauna zooplanctónica. Por lo cual es importante continuar, apoyar y actualizar los estudios centrados en la presencia y diversidad de las especies zooplanctónicas en nuevos sistemas acuáticos.

3. Se detecto por primera vez la presencia invasiva del mejillón cebra en el embalse de La Sotonera, por lo cual el monitoreo constante de los cuerpos de agua es de gran importancia para detectar cambios tanto ambientales como de las comunidades que los habitan.

4. Para la cuenca del Ebro, algunas de las especies que estuvieron asociadas a una baja calidad de agua (eutrofia y potencial ecológico moderado-pobre) y que podemos utilizar como indicadoras son: *A. americanus, D. cucullata, D. brachyurum, B. angularis* y *K. cochlearis*. Mientras que las especies que estuvieron asociadas a una buena calidad de agua (oligotrofia y potencial ecológico bueno o superior) fueron: *D. longispina, A. ovalis y A. saltans.*

5. Las métricas basadas en abundancias y biomasas de los diferentes grupos del zooplancton fueron lo suficiente sensibles para diferenciar entre los diferentes niveles de clasificación, lo cual sugiere que la clasificación de los cuerpos de agua podría ser usada con base a la cantidad de organismos presentes.

6. Las mejores métricas que usaron la abundancia y biomasa del zooplancton fueron: el zooplancton total, la proporción del zooplancton total con la clorofila a, el zooplancton de gran tamaño, los calanoideos, los ciclopoideos, las daphnias y los cladóceros en general.

7. Desde un punto de vista de grupos funcionales, la presencia y bajas abundancias de grandes filtradores como calanoideos y cladóceros pueden ser indicadores de buena calidad de agua. Mientras que la presencia y alta abundancia de copépodos raptoriales como los ciclopoideos indican una baja calidad de agua.

8. Los microcrustáceos en comparación con los rotíferos fueron más sensibles y tuvieron una mayor importancia para la determinación de la calidad del agua, tanto a nivel de grupos funcionales como en términos de abundancia y/o biomasa. Mientras que a nivel de especies los rotíferos fueron buenos indicadores para buena o mala calidad de agua.

9. A través del aprendizaje automático se obtuvieron diferentes precisiones en los modelos para predecir la calidad del agua usando los grupos funcionales. Siendo más precisos los modelos que utilizaron los datos de los diferentes grupos de microcrustáceos que los usados con los rotíferos.

10. Utilizando las abundancias de los grupos funcionales, se establecieron los límites para clasificar los embalses entre cada categoría tanto del potencial ecológico como del estado trófico.

11. Los datos obtenidos del zooplancton en conjunto con los proporcionados del fitoplancton podrían dar mayor visión más completa del sistema, así como de una mejor y más precisa determinación de la calidad del agua. Además de que el muestreo del zooplancton no representa un mayor gasto económico, ya que puede ser obtenido fácilmente de forma paralela al fitoplancton.

12. Finalmente, los resultados de la presente tesis indican que el zooplancton es un buen indicador de la calidad del agua. Por lo tanto, sugerimos que debería ser incluido como un elemento más dentro de la DMA.

General Summary

Access point, sampling boat and
landscape Benagéber reservoir, 2021.

General Summary

Introduction

The aquatic environments and their threats

Water is a fundamental element for life, ecosystems, communities, populations and species existence, including humankind. Approximately 97% of the water on the planet contains high amounts of salt, which can be found mainly in seas and oceans. While only about 3% of water contains none or very low amounts of salt, which is called freshwater. Slightly more than half of this freshwater remains in a solid state as ice and is located at the poles, while the remaining freshwater is found within the continents and is called epicontinental or inland waters. Freshwater is mainly divided into surface and groundwater. Surface water include natural habitats such as rivers and lakes, and modified habitats such as reservoirs.

Currently, all waterbodies are threatened and under different types of pressure. The main pressures result from human activities and the use of water for various purposes. These pressures can lead to several scenarios, such as poor water management, eutrophication, pollution, overexploitation of groundwaters, etc. All these factors can promote a loss of water quality and biodiversity in the environment. In addition to the pressures that are made directly by human activities, waterbodies are subject to the effects of global change, increasing temperatures and more extreme seasons (Woolway et al., 2022).

Among the mentioned water pressures, accelerated eutrophication or anthropogenic eutrophication is one of the most worrying issues today (Schindler, 2012). Anthropogenic eutrophication is due to discharge of wastewater into waterbodies, which increases nutrient loads, mainly phosphorus and nitrogen (Moss et al., 2011). Some of these nutrients may come from dumped waste and fertilizers. Even if they are not discharged directly into a lake, for example, they can be washed into a watershed during rainfall and end up in a waterbody. This nutrient increase can affect the system and can promote the appearance of blue-green algae or cyanobacteria (Paerl & Huisman, 2009). Cyanobacteria can produce toxins (cyanotoxins) affecting other populations that inhabit the ecosystem where cyanobacteria appear and can also put human health at risk. Nutrients increase can not only promote the appearance of these cyanobacteria, but can also increase eukaryotic microalgae abundance and biomass. All these factors can negatively impact water quality, increasing, for example, its trophic state.

Another factor which severely impacts aquatic environments and water quality is global warming. It affects waterbodies in various ways, for example, it has been shown that the duration of the frozen period of lakes has shortened and the ice cover has become thinner (Imrit & Sharma, 2021). While in the lakes where there is no ice cover in any season, it has favored a greater and longer stratification, even in polymictic systems (Cohen et al., 2016). On the other hand, in shallower water bodies, climate change can promote more extreme temperatures and seasonality, thus together with longer droughts, may favor the disappearance of permanent waterbodies, which are re-established only in the seasons when rainfall is abundant (Woolway et al., 2022).

Accelerated eutrophication due to human activities, global warming, and pollution among others, has ecological impacts and consequences. These impacts can bring changes in water quality and aquatic ecosystem services such as fishing and aquaculture. Also, they can affect the structure of trophic webs and the diversity of species that inhabit aquatic environments, such as birds, mammals, fish and microscopic organisms.

Within the communities that inhabit the different aquatic systems, there is one composed of organisms adapted to live in suspension in the water column, i.e., they cannot swim against the current and are bottom-independent. Due to these characteristics, the term "plankton" (drifter) was coined for them. Plankton is made up of small organisms and generally no larger than 5 mm. Depending on their size, they can be classified into different categories. Femtoplankton is composed of viruses and is smaller than 0.2 µm in size. Picoplankton ranges from 0.2 to 2 µm and is formed by bacteria mainly. Nanoplankton is from 2 and 20 µm, includes autotroph and heterotroph organisms such as flagellates, ciliates and small green algae. Microplankton (20-200 µm), is mainly represented by microalgae, whereas mesoplankton (0.2 - 2 mm) and macroplankton (above 2 mm) are mainly represented by different groups of heterotrophic organisms such as rotifers, cladocerans, copepods, larvae, etc. (Finkel et al., 2010; Sieburth et al., 1978).

Plankton is made up of two large groups. The first one is the phytoplankton, composed of algae and cyanobacteria, and both can be unicellular or colonial. These microalgae and cyanobacteria are autotrophic organisms and obtain their energy through the photosynthetic process. Some of the most representative classes are: *Chlorophyceae* (green algae), *Bacillariophyceae* (diatoms), *Dinophyceae* (dinoflagellates), *Cyanobacteria* (blue-green algae), *Phaeophyceae* (brown algae) and *Rhodophyceae* (red algae). Phytoplankton is considered the basic unit of production of organic matter and is the primary producer within the energy web in standing waters. Inside this trophic web, phytoplankton is the main source of energy for the next level, which is also the second main group that composes the plankton: zooplankton.

What is zooplankton made up of?

The zooplankton community is composed of a large variety of heterotrophic organisms from different phyla and classes of invertebrate animals. Zooplankton is made up of heterotrophic organisms that cannot swim against the flow during all or part of their life stages. Additionally, it also includes several larval stages of organisms that later will swim against the flow (Lampert & Sommer, 2007). The groups that compose zooplankton also depend on the environment they inhabit, being very different between marine and freshwater. In the case of freshwater systems, three zooplankton large groups are the most common: rotifers, cladocerans and copepods.

Rotifers are pseudoceolomate organisms with an approximate size between 50 and 2000 µm and have bilateral symmetry. Rotifers present a peculiar characteristic called eutelia, which consists of that a species has a constant number of cells when it reaches maturity, and this number is species-specific. Nowadays, more than 2000 different species have been described and there are records of these animals in practically all aquatic environments, and even on lichens and mosses (Segers, 2008). Three main characteristics define this group, firstly, at the anterior part, they possess a ciliated crown to filter their food as well as for locomotion. Secondly, the presence of a mastax (shredding organ): a muscular pouch containing jointed trophies used to chop and process ingested food. Thirdly, the body can be “naked” or exhibit a hard shell, called lorica. Rotifers have two different reproductive strategies, sexual with the production of males and females, or asexual with only the presence of parthenogenetic females.

Together with rotifers, the microcrustaceans composed of cladocerans and copepods are the dominant zooplankton groups in freshwater environments. The cladocerans are represented by more than 600 species that have been described so far; however, it has been estimated that this number may be much higher (Forró et al., 2008). Cladocerans' size ranges generally from 0.2 to 3 mm, although the species *Leptodora kindtii* can reach 18 mm of body length. The cladocerans have a soft body and are characterized by a bivalve carapace or shield that protects the body but not the head, which is protected by a helmet. The valves are made of chitin and possess four to six thoracic pairs of appendages, which have the function of selecting and filtering the food. The head which is also made of chitin, has an ocellus, two eyes and two pairs of appendages (antennules and antennae). At the end of the body, these microcrustaceans have a postabdomen that they use to clean their filtering appendages. In the same of rotifers, cladocerans have sexual and asexual reproduction, being the asexual reproduction normally the more common.

Copepods are the third dominant group of the zooplankton. They are the largest freshwater zooplankton in terms of body proportions, ranging from 0.5 to 5 mm. Their body has a cylindrical and segmented shape, composed of prosome and urosome. The prosome carries the head with a simple naupliar eye, a pair of antennae and antennules, and the swimming appendages. The urosome includes the remaining thorax somites plus the abdomen and ends in a pair of caudal rami with two caudal setae. Unlike the other two groups, this group only presents sexual reproduction with the production and fertilization of eggs. Copepods during all their life pass through different larval stages, the first being the nauplius form, to later become copepodites and finally their adult form. Compared with the other zooplankton groups, they are the group with the greatest diversity in species number. More than 10,000 species have been identified, most of them live in marine environments, while in freshwater more than 2,800 species have been reported (Boxshall et al., 2007). Among the freshwater species, the great majority belong mainly to three orders, Calanoida, Cyclopoida and Harpacticoida.

Finally, in some freshwater environments, the larval stage of several animal groups (i.e., mollusks and fish) show a planktic lifestyle, and dominate zooplankton. One example of this is the zebra mussel veliger larvae, which can have a very high abundance and biomass in water bodies compared to the rest of zooplankton community. This can be seen in different regions, as is the case of reservoirs in the Ebro basin (Durán et al., 2011; Lalaguna & Marco, 2008). In the case of saltwater environments, different groups such as trochophores and fish larvae can be more dominant under certain conditions than micro crustaceans.

The role of zooplankton in aquatic environments

Zooplankton communities play a very important role in aquatic environments in many ways. As members of the trophic web, they facilitate the flow of energy between primary producers (e.g., microalgae) and secondary consumers like fish. A common way to simplify the energy web is as follows: phytoplankton, zooplankton, small planktivorous fish and piscivorous fish. However, there are many more issues involved, such as detritus or dissolved and particulate organic matter. Detritus can become more abundant than phytoplankton in some periods and it can eventually integrate zooplankton diet (Matveev & Robson, 2014). When the availability of detritus is higher, it would favor the detritus-consuming species from the next levels. In this case, the heterotrophic bacteria, ciliates and flagellates from the microbial loop can also become a great energy source for zooplankton (Azam et al., 1983; Taipale et al., 2012; Taipale et al., 2014). This is because some zooplankton species can filter and consume those organisms, thus interacting and integrating the microbial loop into food web at a higher level (Moshe & Geller, 1984).

Normally has been said fishes are the next level within the trophic web consuming zooplankton, however, fish are not the only predators (Vanni, 1988). Aquatic insects and even other zooplankton species such as cyclopoid copepods, some rotifers and the cladoceran *Leptodora kindtii* are active predators of other member of the zooplankton (Leoni, 2017). All these interactions with carnivorous zooplankton that is also consumed by fish, promote the flow of energy and carbon in different ways forming an interactive multidimensional web (Kruk et al., 2021), which further increases the importance of zooplankton in aquatic environments.

Another function of zooplankton is their role in top-down control of phytoplankton (Carpenter et al., 1985). This control is performed by zooplankton pressure over phytoplankton and this pressure can shape the structure and dynamics of algae community (Jeppesen et al., 1997; Naselli-Flores & Rossetti, 2010). For example, when the filtering zooplankton species increase in numerical abundance and diversity, this could increase the grazing pressure, controlling algal density of edible species, and leading to higher water transparency. Conversely, zooplankton can be adversely affected during bottom-up control events due to phytoplankton density increase or decrease. When algae density decreases during winter season in temperate regions, zooplankton density normally decreases too (Sommer et al., 1986). Otherwise, when phytoplankton rapidly increases abundance, e.g., in a bloom, zooplankton can also respond quickly increasing their density shortly after algae increase (Sterner & Paul, 2009). Thus, what happens when phytoplankton density grows without control? This uncontrolled growth can promote several changes in the environment, like an increase in organic matter and turbidity, among others. Higher turbidity limits the light penetration in the water column affecting different communities, such as macrophytes (Austin et al., 2014). Therefore, the control performed by zooplankton over phytoplankton and their importance in energy transfer, make zooplankton a key element inside the aquatic environment.

Water Framework Directive and zooplankton exclusion

Due to the threats and problems that waterbodies face around the world, some measures have been taken in different countries and regions to mitigate water and habitat degradation. In the case of the European Union, in October 2000 the Water Framework Directive (WFD) was established as a part of its water management policy (Directive 2000/60/EC). The main goal of the directive is to achieve good ecological status for all its waterbodies, including lakes and similar artificial or heavily modified waterbodies (reservoirs) larger than 50 hectares. Currently, this directive presents and establishes the methodological guidelines to be followed by EU members states and how to classify the ecological status of their superficial waterbodies.

In the guidelines, the WFD indicates the different classification categories for the estimation of waterbodies´ ecological status, or in the case of reservoirs, the ecological potential. This classification is represented by the next categories or levels: Good or higher, Moderate, Poor and Bad. To estimate the ecological potential, the directive has established the variables to be measured in each waterbody which are split into biological and physical and chemical parameters. The following physicochemical variables are considered: Secchi disk transparency, dissolved oxygen concentration and nutrient concentration via total phosphorus. All these variables are strongly correlated to biological productivity, which indicates the system's capacity to transform energy into organic matter. High amounts of nutrients can promote and accelerate the eutrophication process.

The biological variables or biological groups that were chosen to be included within the WFD criteria were called Biological Quality Elements (BQE) and published in annex V. These BQEs include several and diverse groups or organisms that possess different life cycles, strategies, habitats, reproduction forms and sensitivities to changes inside aquatic environments. Several groups were chosen including fish, macroinvertebrates, aquatic plants, phytoplankton and phytobenthos. However, surprisingly zooplankton was not included within WFD and its exclusion elicited a big controversy since was not given further explanations (Caroni & Irvine, 2010; Erik Jeppesen et al., 2011; Moss, 2007).

Zooplankton probably was not included since its relations with eutrophication processes were not fully studied and are not so directly related to growth as for phytoplankton. However, their exclusion was not detailed or explained in the official documents (Caroni & Irvine, 2010). As I stated previously, zooplankton is a key element inside aquatic environment. They can shape phytoplankton communities, are important in energy transfer, and are also sensitive to changes in the environment and could be good BQE for this directive (Jeppesen et al., 2011).

Several studies worldwide have shown that zooplankton species are extremely sensitive to the changes experienced by the freshwater systems where they inhabit. Most of the studies were performed using rotifers, copepods and cladocerans as model organisms. Furthermore, it has been indicated that several species and groups are good indicators of water quality (Almeida et al., 2020). The zooplankton community is related to physical and chemical variables, and some species are very related to those variables that determine the trophic status such as nutrients, chlorophyll a and water transparency. Thus, some species have been used as indicators of trophic state and linked to its categories: oligotrophy, mesotrophy and eutrophy (Kehayias & Doulka, 2014; Obertegger & Manca, 2011; Paes De-Carli et al., 2019).

The number of studies focusing on zooplankton as an indicator of the ecological potential of aquatic ecosystems is lower than that addressed at determining the trophic status of these environments. However, all those studies are a good and solid base to point out zooplankton efficiency as indicators. Even though initially the WFD has left out zooplankton as BQE, there is not a rule that says that cannot be incorporated later. Therefore, studies devoted to research their use as tools to determine ecological potential, together with steps to follow or methodology to use to its incorporation into the WFD are needed.

To fulfill this need, this thesis was created and as a research response to counteract the exclusion of zooplankton community as one of BQE within the WFD. Therefore, the present study intended to determine if zooplankton could be a suitable tool as a water quality indicator, especially for ecological potential and trophic status in freshwater habitats. For this purpose, I use different approaches and models, such as taxonomic characterization of the zooplankton community at species and group level, metrics associated with abundances and biomasses and finally the use of machine learning and functional groups. To achieve this, I use a robust dataset from samples collected over a long term from multiple reservoirs with a wide spectrum of water quality containing information about the zooplankton communities from 66 reservoirs located in the Ebro watershed in Spain.

Water quality assessment using zooplankton, a mini review

In this section, I would like to give a brief explanation of how and when zooplankton has been used to determine water quality. The following research examples could be mentioned in published chapters, used barely in the discussion section of them, or not mentioned. Hence it is important to know if previous work could provide valuable information in the goal to include zooplankton within the WFD context.

In the last 20 years, the number of research studies using or testing zooplankton as a water quality indicator has been increasing. This is mainly because of zooplankton's importance has been recognized in aquatic systems and its sensitivity to changes in the environment. The increased number of publications on this subject has implied the creation of several indices that have been developed in different parts of the world. These diverse indices use different elements of zooplankton as model. Some of them use only selected groups, such as rotifers, copepods and cladocerans; others use the whole community. A high number of studies and indices used zooplankton to evaluate the trophic state of different waterbodies. Nevertheless, studies focused on determining ecological potential within the WFD using zooplankton community are scarce compared with those related to the trophic state.

Some studies using or testing zooplankton have in common that they have been developed using only one or a few number of waterbodies. For example, the Lake Erie in the United States, where some phytoplankton and zooplankton metrics were tested to assess the trophic status of the lake (Kane et al., 2009). Crossing to the other side of the Atlantic Ocean, in lake Pełcz in Poland, zooplankton community species were used to assess trophic state (Piasecki & Wolska, 2001). Also, in the United Kingdom, in Loch Lomond, only rotifers data was used to determine that species abundances varied along the trophic gradient (May & O'Hare, 2005).

Other studies that were performed only in one waterbody, have a good record of data over time, these records may be years or decades. This is the case of a study conducted in central Estonia, where different species from the whole zooplankton community were obtained as indicators of eutrophy and oligotrophy (Haberman & Haldna, 2014). On the other hand, in the southern part of Brazil, an index was developed using species from three zooplankton groups (Paes De-Carli et al., 2019). The elaboration of this index was made for reservoirs and to determine their trophic status and make future predictions on it, however, only samples from seven different reservoirs were used. All these previous works and indices, created with data from one waterbody or the one developed in seven, still have limitations for not having a high representation of different types of waterbodies, grades of eutrophy and communities. Therefore, they may only be applied to the same waterbody or region from where they developed.

Other studies have used a higher number of waterbodies. For example, the Wetland Zooplankton Index (WZI) was developed using 70 reference lakes as reference in the Great Lakes basin area, between United States and Canada (Lougheed & Chow-Fraser, 2002). A strong point in this work is that several zooplankton species were classified as water quality indicators for different levels. However, the authors indicated that the use of this index should be only applied in lakes from the same regions and conditions. Besides, they mentioned that other methods, techniques, and approaches should be considered to not depend only on a species approach.

In the previous studies, most of these indices were created using the presence or absence of certain species. But, what about zooplankton´s numerical abundance? The number of studies using this element is small, yet we can still find some studies that employ it. For example, a robust study using data from more than 70 lakes in Poland was conducted using zooplankton abundances. In two similar works were obtained indices with different formulae and metrics to determine trophic status using rotifers (Ejsmont-Karabin, 2012) and microcrustaceans (Ejsmont-Karabin, 2016). Subsequently, in 16 Greek lakes, these indices were tested and a new index was proposed using total zooplankton community abundance (Stamou et al., 2019). The three studies inferred zooplankton abundances, in total or split by groups, were useful to differentiate and determine trophic status in lakes. Although these studies used data from several lakes, due to their aims, indices to determine ecological potential were not evaluated or proposed. Thus, they left aside useful information that could be used for the inclusion of zooplankton in the WFD.

As I stated previously in this thesis, the studies testing zooplankton as an indicator of ecological potential of a waterbody are less numerous than those addressed at inferring its trophic status. But, among the few works assessing ecological potential within freshwater environments, we can find two recent studies. The first one was carried out again in Poland and proposed the Zooplankton Index for Polish Lakes' Assessment ($ZIPLA_S$). This index was created across 45 different lakes using different metrics focused on species, ratios and abundances (Ochocka, 2021). Based on this new index, several species associated with different trophic levels were identified. One novelty is that the new index sets up boundaries or thresholds to determine the ecological potential classes. In comparison with the rest of studies, the ZIPLAs index is quite complete, with improvements in a few areas, such as the implementation of functional groups and machine learning to be more robust.

The second study, located in northern Portugal, used species and functional group-level approach (Almeida et al., 2020). Although this study was conducted during different seasons throughout the year, it has a limitation related to have used data from only four different reservoirs that were relatively close. In addition, these reservoirs did not have a representation of low water quality systems and most of the time reservoirs were classified as good water quality. However, these studies indicate that zooplankton groups can be used as a tool to determine ecological potential within the WFD context.

Moving closer to our study area, a couple of studies used zooplankton communities to assess both trophic status and ecological potential (García-Chicote et al., 2018; García-Chicote et al., 2019). These were made in the Jucar watershed and included data from 20 reservoirs during the summer and winter seasons over four years. One of these studies was able to identify different species as indicators of low water quality, but not as indicators of good water quality (García-Chicote et al., 2019). This lack of good water indicators could make it difficult in the future to implement an index or protocol to determining oligotrophic environments. The second research detected an increment in both abundances and biomasses within eutrophication and moderate-poor levels (García-Chicote et al., 2018), indicating that zooplankton could be used as a tool for water quality indicators.

In the past, the Ebro Watershed Confederation developed the Zooplankton Reservoir Trophic Index (ZRTI) for reservoirs located in the study area considered in this thesis. This index tested zooplankton species to determine reservoirs´ ecological potential using a multivariate analysis approach. However, its application is somewhat limited as it only evaluates the position of species placed in a CCA (Montagud et al., 2019). Additionally, this index does not consider other traits, such as abundances and functional groups, and these information gaps are an opportunity to test and develop new strategies.

Having a larger amount of data opens the door to perform a better and more robust data analysis, for more valuable results and the creation of more reliable indices. Yet in general, we observed that some previous studies used data from one or a few lakes or reservoirs to propose zooplankton indices. Not too many studies had information collected from more than 10 lakes or reservoirs. But, why do we have this lack of information from aquatic systems? This is mainly due to a lack of budget for projects, although, large projects or monitoring programs are vital to obtain good and precise information from freshwater environments over time. These programs should be implemented not only from a scientific view but also from a governmental interest. Thus, having a robust program monitoring water quality for years in a higher number of waterbodies is beneficial to the society. Under one of those large projects, this thesis was made.

Therefore, one strong point of the present thesis is the robust dataset that was obtained over time with more than 300 samples. Unlike other studies, this dataset has representations of reservoirs classified in all categories of both, trophic status and ecological potential. This gives us a wide spectrum of reservoirs' physical and chemical variables and how them varies within the water basin. This information can lead us to know which zooplankton species that inhabit reservoirs are related to environmental variables and which species and metrics can be good water quality indicators. Furthermore, the different approaches from all the previous studies could be considered as a reference point for the present thesis. So here, we are retaining the useful information and improving the lack of points and trying to fill the gaps in the use of different approaches.

Thesis Objectives

Thesis Objectives

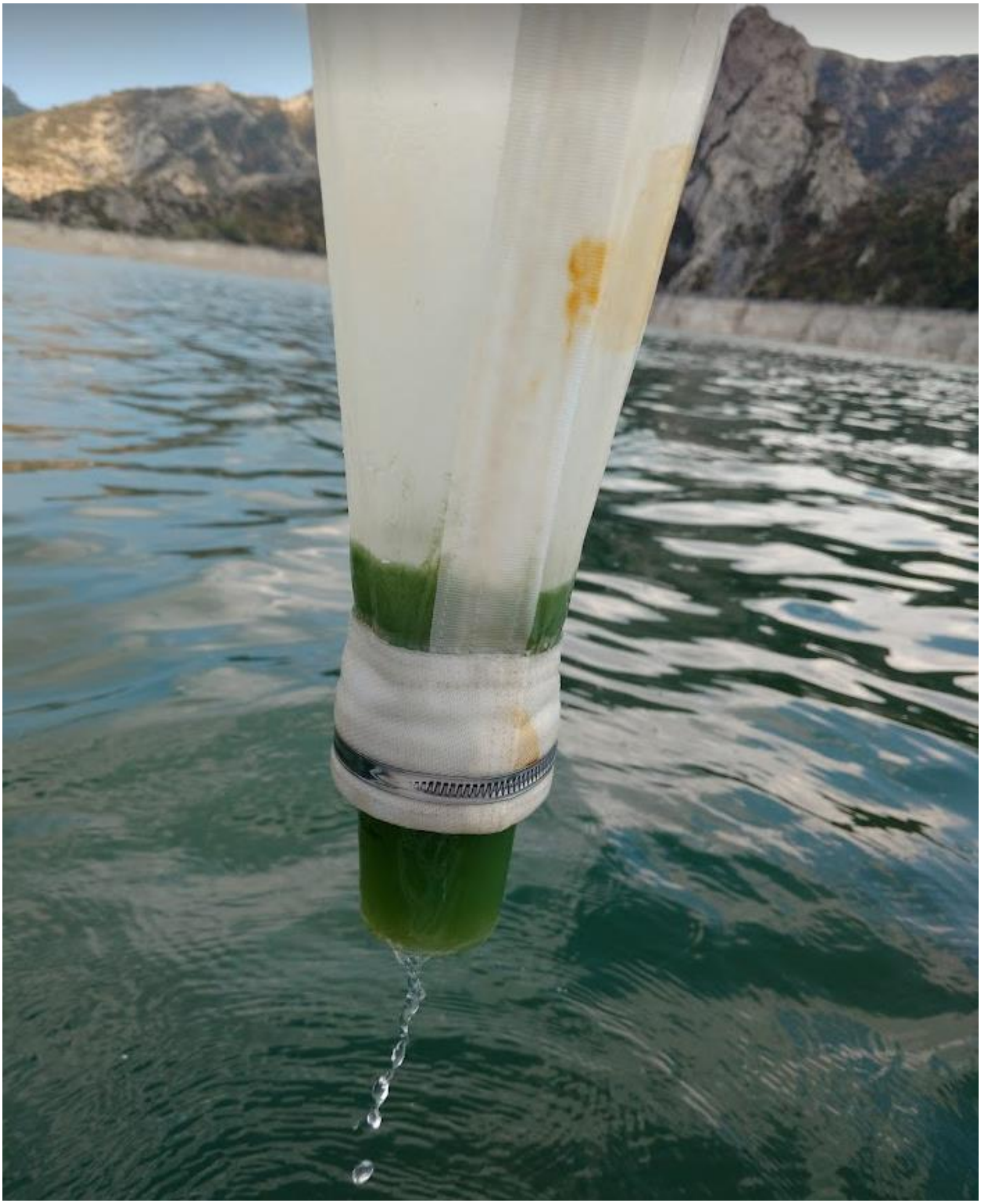
The main goal of the current doctoral thesis is:

- Evaluate if zooplankton communities are good indicators to determine water quality in Ebro´s reservoirs. This is done through different approaches: identification of species present, zooplankton groups numerical abundance and biomass, use of zooplankton functional groups and machine learning.

Once this main goal has been established, the specific goals are:

1. Analyze the changes in species composition in six reservoirs during two different seasons (summer and autumn) and determine which environmental variables are responsible for these changes.
2. Indicate which zooplankton species of major groups (rotifers, cladocerans and copepods) can be used as indicators of trophic state and ecological potential in Ebro´s reservoirs.
3. Determine if zooplankton group´s abundances and biomasses may be used to differentiate among the trophic state and ecological potential levels in the studied reservoirs.
4. Determine reservoirs´ water quality using a functional group-traits approach and the implementation of predictive models with machine learning.

Material and Methods



Vertical zooplankton net with high phytoplankton biomass.
Oliana Reservoir, 2019

Material and Methods

The methodology used to obtain data from the current thesis was organized based on their purpose and separated into several sections. The main section refers to methodology applied in reservoirs during every sampling campaign throughout all years. There are specified steps and procedures to follow to obtain the environmental data inside every reservoir. Environmental data included of physical and chemical variables from the water column. Also, was obtained some morphologic data (reservoir water volume, percentage of water in the reservoir, maximum volume, maximum area, maximum depth, etc.) related to sampling day from the Ebro´s Confederation website (https://www.chebro.es). The main section also indicates how to collect and to properly preserve the biological samples. These samples were composed of phytoplankton and zooplankton. Despite the objectives of the current thesis, was not use phytoplankton as an indicator, its abundances and biomasses were used as another variable for zooplankton analysis. In addition, it is specified how was identified zooplankton species and how both, trophic status and ecological potential was determined in each reservoir.

Here is described for every different thesis chapter the methodology used for data analysis based on their particular goals. Once all data from the field were obtained, different matrices were created with data from all reservoirs: i) environmental variables matrix (physicochemical and morphologic data) ii) zooplankton species, abundances and biomasses. iii) phytoplankton species and their respective abundances and biomasses.

General methodology

The Ebro´s watershed flows across nine different autonomous communities (Cantabria, Castilla y León, Castilla La Mancha, La Rioja, País Vasco, Navarra, Comunidad Valenciana, Aragón and Cataluña). Is the biggest basin in Spain with more than 86.000 km^2 and due to its high extension, it is one of the major watersheds in the Mediterranean region. With that in mind, in general for the current thesis, presents a high representative data obtained from 66 reservoirs located along the Ebro watershed during the summer season from 2010 to 2019.

Inside each reservoir, a sampling point was set up at the deepest part and located a distance of 300 to 500 meters away from the dam. In this sampling point, several environmental variables were measured (chlorophyll a, temperature, dissolved oxygen, total phosphorus, total nitrogen, Secchi disk transparency depth, turbidity, water column depth, total suspended solids, conductivity, ammonium alkalinity, etc.) and phytoplankton and zooplankton samples were collected. For a complete list of physical, chemical and morphologic data variables measured see the published papers which are part of this thesis. Trophic state was determined for each reservoir using the Trophic State Index (Carlson, 1977). Ecological potential was obtained following methodology within the "'Spanish Legislation RD 817/2015'' and WFD (Directive 2000/06/EC). This directive uses physical and chemical and algae variables to determine the water quality. As physical and chemical variables, Secchi disk depth, hypolimnetic oxygen concentration and total phosphorus were used. The algal variables included chlorophyll a concentration, biovolume, percentage of cyanobacteria and the Index of Algae Groups (IGA) (Catalan and Ventura 2003).

To collect zooplankton samples two different methods were employed. At first, a vertical town was taken with a net of 45 µm mesh size Nytal. The net was towed from 30 m depth or from the reservoir bottom (in case depth was less than 30 m) until the surface. This vertical net was used only as a reference and to qualitatively assess the whole zooplankton community present in the reservoir in details to facilitate the taxonomic purposes. Later on, quantitative data were obtained using a vertical Ruttner bottle with a 2.7 liters capacity. This bottle was used two times, to obtain 5.4 L water sample in total. The depth at which zooplankton samples were collected was at the oxycline beginning, this zone has been reported as the richest zone of zooplankton (Miracle & Vicente, 1983). Then, the water sample from Ruttner bottle was filtered through a 30 µm mesh size Nytal. Afterwards, both, vertical tow and Ruttner bottles were stored in hermetic glass vials and preserved with 4% formalin. Phytoplankton samples were obtained also using a vertical tow of 25 µm mesh size and Ruttner bottles. Phytoplankton numerical abundance and biomass were used as other variables to understand relations with zooplankton community.

Then, in the laboratory, Ruttner samples were counted using a Sedgewick Rafter-type chamber (1 mL) to obtain species richness and abundance. Zooplankton species were identified under an inverted microscope (Nikon Eclipse Ti-U) using specialized literature for every zooplankton group. Microcrustaceans were identified using Alonso (1996) and Błędzki and Rybak (2016). In the case of rotifers, they were determined with guides of Ruttner-Kolisko (1974), Koste (1978), Nogrady et al. (1995) and Nogrady and Segers (2002).

Methodology by thesis chapters

In the first chapter of this thesis, an ecological approach was used to investigate zooplankton communities. Here, as a screening study, was examined if there were differences among communities using only data from six reservoirs with different water quality and locations during summer and autumn seasons and which environmental variables were related to these changes. For this purpose, was used diversity indices, Analysis of Similarity (ANOSIM) and Similarity Percentage analysis (SIMPER). Also, was ran the multivariate Canonical Correspondence Analysis (CCA) from two different perspectives. First, to determine the relationship between environmental variables and zooplankton species and second, to verify environmental variables and zooplankton major groups.

After I obtained a general idea of community changes that occur in reservoirs with different water quality, the next goal was to find those zooplankton species that could be good indicators for ecological potential and trophic status for reservoirs. So, with this idea in mind, in the **second chapter**, I created the above-stated matrices, which included data from all sample occurrences. As in the previous chapter, I used SIMPER and CCA analyzes. The first one was used to determine those species that were responsible for community changes. The second was used to check the relationship between the environmental variables and zooplankton species, especially those physicochemical and morphological variables that determine water quality. In the last part of this chapter, I ran the Indicator Value (IndVal) method using species occurrence and abundance data to find ecological potential and trophic status indicator species. All these statistical techniques were carried out using the R programming language (R Core Team, 2021).

Once I obtained those indicator species; I wanted to move forward and verify if other elements of zooplankton were suitable for water quality assessment. Hence, in **chapter three** several metrics based on literature and personal observations were proposed. The metrics were computed based on zooplankton abundances expressed as individuals per liter (ind L^{-1}) and biomass estimated as dried weight per liter (DW L^{-1}). Thus, the proposed metrics were a) ZOO (zooplankton total community), b) LZOO (large zooplankton), c) SZOO (small zooplankton), d) ZOO:CHLA (zooplankton: chlorophyll a ratio), e) ZOO:PHYTO (zooplankton: phytoplankton ratio), f) Zooplankton major groups, g) Selected microcrustacean orders/genera: calanoids, cyclopoids and daphnids. Pearson correlations and multiple regressions were performed to calculate the relationship between proposed metrics and environmental variables. A t-student test was carried out to check if the metrics had significant differences among trophic status and ecological potential levels. Also, this test was used to indicate which metrics could be used as indicators or could differentiate among water quality levels.

Finally, for **chapter four**, a functional groups approach and the implementation of machine learning models was used. In this last chapter, to obtain the zooplankton functional groups (ZFG), I selected several functional traits: adult body weight, habitat, trophic group, reproduction form and feeding strategy. Once all functional traits from our data and specialized literature were obtained (Barnett & Beisner, 2007; Barnett et al., 2007; Obertegger et al., 2011) a new species and traits matrix was created with numerical and categorical data. Using these data, a dissimilarity matrix was created using Gower´s method (Laliberte & Legendre, 2010) and then a hierarchical clustering analysis was performed to assign species into functional groups. The environmental and phytoplankton´s numerical abundance and biomass data were used to analyze these functional groups. Once all the matrices were ready, several multivariate analyzes such as Pearson correlations, Principal Component Analysis (PCA) and Redundancy Analysis (RDA) were performed to identify those variables that influenced the functional groups and that could shape them.

The environmental variables and functional groups matrices were used to determine ecological potential and trophic status through a machine learning approach. The machine learning model chosen to predict water quality was Random Forest (RF). This method has been proven as a powerful and robust technique to analyze ecological data using many de-correlated decision trees (Cutler et al., 2007). Two different RF were run to indicate which functional groups were the most important to determine ecological potential and trophic status. Thereafter, different models were built to predict water quality using ZFG data. These models were created by splitting data in two groups, 80% of the data was the first group that was used for the training model and 20% was left as second group for validation. Finally, the classification and regression trees (CARTs) were used to establish and know the boundaries or thresholds between different categories of trophic status and ecological potential using ZFG abundances.

Results and Discussion

Inverted microscopy for species identification and counting
Laboratory of Limnology, Departament of Microbiology and Ecology
Valencia University, 2022.

Results and discussion

Ebro reservoirs, trophic state, ecological potential and their environmental variables

After ten years of sampling a large dataset of 304 samples were obtained from 66 reservoirs with a wide range of water quality level and zooplankton communities. Every sample was composed of physical and chemical and morphological variables previously established together with plankton samples. The identification of each sample was based on the reservoir name and year sampled, e.g., Llauset 2018. Due to logistical, budget, weather, and technical constraints, not all reservoirs were sampled every year. However, each reservoir was sampled at least every three to four years. As I stated before, compared to other studies, the number of sampled reservoirs and obtained samples with complete environmental and zooplankton data is high. Several previous studies have used a single waterbody as a model, many others used have less than a dozen, while very few exceed fifty different waterbodies.

The Ebro basin area possesses a diverse and varied landscape and morphology, which affected and conditioned the reservoirs at construction, hence the high diversity in the reservoir size and depth. Concerning size, the smallest ones were those that did not exceed one cubic hectometer in volume as in the case of Utexa-Seca. While the largest ones reached a volume of more than one thousand cubic hectometers such as Mequinenza. In the case of depth, it ranged from the shallowest ones of about two meters deep, to the deepest ones that were between 70 to 120 meters deep.

Reflecting the reservoirs' morphologic variables, was recorded wide ranges of values for the physical and chemical variables within the reservoirs. From all the variables measured, the most important were the following: chlorophyll a concentration, which ranged from 0.4 µg L^{-1} to 51.9 µg L^{-1}, total phosphorus, ranging from 0.65 µg $L^{-1}$to 186 µg L^{-1}, and Secchi disk depth, which varied from 0.23 meters to 18 meters. The list was completed by water surface temperature with values from 10.3°C to 28.1°C and dissolved oxygen from 2.5 mg L^{-1} to 14.38 mg L^{-1}.

Chlorophyll a, total phosphorus and Secchi disk depth are proxies of phytoplankton abundance and are worldwide accepted as variables that indicate the water quality in lentic systems. So, based on these three variables, was determined the reservoirs´ trophic status using the Trophic State Index (Carlson, 1977). The results indicate that most of the reservoirs` samples were classified as oligotrophic or mesotrophic since each of these categories had 123 samples. While those reservoirs samples classified as eutrophic or hypereutrophic were 55 and 3 respectively. In addition, was determined the ecological potential following the guidelines established by WFD. To achieve this, was used total phosphorus, Secchi disk, dissolved oxygen and phytoplankton community. It was found that 99 samples were classified as good or higher, while 202 samples were classified as moderate. Finally, only three samples were classified as poor, and none was classified as bad.

Zooplankton ecology

The **first thesis chapter** was established as a screening study, focused mainly on zooplankton ecology. Thus, this chapter reports on the study of only six reservoirs during the summer and autumn seasons to unravel zooplankton community relationships with environmental variables and how zooplankton communities were composed and the species changes inside reservoirs. In the studied reservoirs, 40 different species were recorded from the three major groups (rotifers, copepods and cladocerans) and the invasive zebra mussel larvae *Dreissena polymorpha*. In Spain and its different watersheds, several studies and research groups have obtained and identified the zooplankton species in different waterbodies (see among others: Miracle & Vicente, 1983; De Manuel, 2000; Badosa et al. 2010; Rojo et al. 2012; García-Chicote et al. 2018; García-Chicote et al. 2019). However, the present research presented for the first time the species composition in the Ullivarri-Gamboa reservoir. The species were the cladocerans *Bosmina longirostris, Ceriodaphnia pulchella, Daphnia cucullata, Daphnia longispina*, the Copepod *Acanthocyclops americanus*, the rotifers *Anuraeopsis fissa, Brachionus angularis, Conochilus unicornis, Hexarthra oxyuris, Keratella cochlearis, Keratella cochlearis tecta, Polyarthra dolichoptera, Polyarthra major, Synchaeta pectinata* and the zebra mussel *D. polymorpha*. This indicates that there are still many waterbodies where zooplanktonic fauna is not known. Hence, studies focused on species diversity in new waterbodies should be continued and supported.

Under a zooplankton group-level and species composition approaches using CCAs, was detected that microcrustaceans were strongly related to variables that determine trophic status as previously reported for other reservoirs in the basin (Montagud et al., 2019). For example, cladocerans were related to chlorophyll a amount, indicating that this group can be very sensitive to changes occurring in phytoplankton biomass and copepods were related mainly to Secchi disk transparency. The copepod calanoids species were composed of *Copidodiaptomus numidicus* and *Eudiaptomus vulgaris*, while *A. americanus* was the most dominant cyclopoid copepod. In the case of the calanoid *Neolovenula alluaudi*, its presence in one of the studied reservoirs had been recorded for decades (De Manuel & Jaume, 1993), but curiously was not detected during the present study. This could be due to multiple reasons, such as fish predation (Hansson et al., 2004; Jeppesen et al., 2004), displacement by another exotic species, season of the year when samples were obtained and/or changes in the environmental conditions that may have affected their populations (Devetter, 1998; Dodson et al., 2009). Therefore, to know the changes in the communities through time, constant waterbodies monitoring should be done. Also, this will help to update the taxonomic lists and records that are outdated for many waterbodies, this will unravel more zooplanktonic diversity at a local, regional and country level.

It is worth highlighting that in this chapter was detected the presence of the invasive zebra mussel (*Dreissena polymorpha*) for the first time in La Sotonera reservoir, which previously was free of this invader. Originally from the region between the Black, Caspian and Aral Sea, this exotic species has spread throughout most of the world and has been displacing native species (Minchin et al., 2002). It is currently classified as one of the most harmful exotic species in the world (Lowe et al., 2004). In the Ebro basin, as in many other regions, it not only causes ecological problems but has also been reported to cause economic losses (Durán et al., 2011). Inside the dam and reservoir structures, large populations of zebra mussels have been found obstructing the water flow. This causes a great expense in time and money for their removal, in addition to maintenance work. It is believed that the main means of propagation of this mussel within Ebro´s reservoirs is made by humans. Especially fishermen and tourists, who navigate inside waterbodies without proper boat disinfection and equipment that was in contact with water, thus spreading the larvae (Lalaguna & Marco, 2008).

The results analyzed in the first paper gave me an idea of how zooplankton species were distributed in reservoirs across Ebro´s basin and how these species were related to environmental variables. This study provided us with valuable information and helped to develop a better plan for the remaining studies in the thesis chapters. In addition, results suggest that the knowledge of zooplankton species and the change in their composition through time can be a tool for reservoirs and basins management. For example, a quick and early detection of invasive species, can give time to apply the necessary measures to prevent their propagation and for the implementation of strategies for their control or removal if needed.

Indicator species

After the screening study presented in the first chapter, I obtained a general idea of the composition of the zooplankton communities inside a few reservoirs, and which environmental variables were related to them. A large dataset with all the information available from the previous sampling and ongoing campaigns was created. The last campaign was done in 2019, since in 2020 there was the covid outbreak, implied the stopping the field sampling campaigns and university access. For this reason, it was decided to use all data from previous years (2010-2019), as a matrix model and if possible, in the future validate it with further data. The total zooplankton species richness from all sampled reservoirs was 169 species. Rotifers were the group with the greatest number of species with 115 species in 36 different genera. Rotifers were followed by cladocerans represented by 36 species in 15 genera, while copepods had only 17 species in 11 different genera. The Rotifera genera with the greatest number of species were *Brachionus, Lecane, Polyarthra, Synchaeta* and *Trichocerca*. On the other hand, the most representative microcrustaceans genera were *Daphnia* for cladocerans and *Cyclops* for copepods.

Species from several aquatic groups, e.g., fish, aquatic plants and phytoplankton, have been used as indicators of water quality in different studies conducted in different regions around the world (Carlucci et al., 2018; Catalan et al., 2009; da Silva, et al., 2014). Although different methodologies have been established for each group, generally, species presence associated with high or low water quality is broadly used. Using a similar approach, the **second chapter** of this thesis was developed. Thus, using different data analyses were reported 14 species that were classified as good indicators for trophic status. In the case of ecological potential, indicator species were reduced to only six. This suggests that zooplankton species are more sensitive to discerning among trophic state levels than ecological potential. This may be because thresholds among trophic status are more defined than in the ecological potential.

Previous studies using zooplankton species as water quality indicators showed that cyclopoid copepods (Duggan et al., 2020; García-Chicote et al., 2019; Perbiche-Neves et al., 2021; Perbiche-Neves et al., 2014) together with rotifer *Keratella cochlearis* f *tecta* (Duggan et al., 2001; Haberman & Haldna, 2014) are indicators of eutrophic conditions.

Our results were similar since, through IndVal analysis, the rotifer *K. cochlearis* f *tecta* and the cyclopoid *A. americanus* were selected as indicator species or associated with eutrophy and poor ecological potential. In addition, these species were strongly related to chlorophyll a, total phosphorus and turbidity in the CCA. Generally, high valuables of these variables were indicative of eutrophy or low ecological potential. Furthermore, was found that rotifers *Collotheca pelagica, B. angularis, Pompholyx sulcata* together with the cladoceran *D. cucullata* are species that indicated low water quality in our study, as they were previously reported as eutrophic species (Haberman & Haldna, 2014; Kehayias & Doulka, 2014; Smakulska & Górniak, 2004).

Contrary to these low water quality indicator species, one species associated with oligotrophy and good ecological potential was found. This species was the cladoceran *D. longispina*. Additionally, *Ascomorpha ovalis* and *Ascomorpha saltans* were indicators of oligotrophy. Even though the results only show three indicator species, it means a good advance, since under a similar approach other studies could not define any species related to oligotrophic conditions (García-Chicote et al., 2019). Also, small-size species like rotifers *P. dolichoptera, P. major, A. ovalis, K. cochlearis* and the mussel *D. polymorpha* were detected to be responsible for changes in species composition inside Ebro´s reservoirs. This was similar to the findings in the first chapter. However, in this case a higher number of reservoirs was compared and this finding contrasted the highest level of both, trophic status and ecological potential with the lowest. This indicates that these small-size species have high importance in aquatic environments, especially when they replace large filter-feeding species as the trophic state increases (Jeppesen et al., 2000; Pinto-Coelho et al., 2005). This replacement of species with different sizes and which belongs to different functional groups along the trophic gradient also provides a possibility to test them as water quality indicators.

Currently, the only planktonic species used as indicators to determine ecological potential within the WFD are those belonging to phytoplankton community. However, our results suggest that zooplankton species could be good water quality indicator for Ebro basin reservoirs. It is important to test these species should be tested in other regions and waterbodies such as lakes to verify their efficacy as indicators. If so, then in a near future, after a review, zooplankton indicator species could be included in WFD.

Abundances and biomasses as bioindicators

If count the numerical abundance or number of organisms present along several waterbodies, it can be noted that there is a big difference between aquatic environments with different trophy states. This abundance or density is conditioned by the quantity and quality of available resources to maintain different populations i.e., the carrying capacity of the habitat. Normally in inland waters, the limiting nutrient for phytoplankton is phosphorus (Lampert & Sommer, 2007). Oligotrophic waterbodies contain low amounts of phosphorus, while in eutrophic waterbodies this resource is much more abundant causing an increase in microalgae abundance. As phytoplankton community becomes more abundant and available as food, zooplankton soon also increase their abundance. So, a high plankton abundance means a higher trophic state and generally a low water quality. Could we determine reservoirs' water quality using zooplankton abundances? Under this question, the **third thesis chapter** was proposed.

Then, to try to resolve this question was measured zooplankton abundances for every reservoir and species, but I also wanted to review if another metric could be useful for our research. So, biomass was calculated for every species. As in the previous chapter, due to reservoirs' great diversity in both morphometric and environmental variables, large differences were found among the highest and lowest values of abundances and biomasses. For example, the lowest total zooplankton abundance was only 6.76 ind L^{-1}, while the maximum reached 2758 ind L^{-1}, whereas biomass had minimum and maximum of 0.45 µg DW L^{-1} and 1971 µg DW L^{-1}, respectively.

To analyze zooplankton community, different metrics were used as it was mentioned previously in the methodology section (complete community, by major groups, ratios with phytoplankton and chlorophyll a, microcrustaceans orders and genera). In general, it was noted that most metrics had a positive tendency to increase their numbers as eutrophication increased. Also, when comparing trophic state and ecological potential, the former showed a stronger positive increase. The metrics that did not show a marked positive trend were SZOO and ZOO:PHYTO, while ZOO:CHLA had a negative trend, due likely due to a major pressure exerted by zooplankton on phytoplankton, as a result of the increased zooplankton abundance and biomass (Gyllström et al., 2005).

One of the major WFD interests is to know the actual ecological potential of their waterbodies. For this, it has used several metrics or BQEs as was mentioned before. Also, another interest of this directive is to have tools to differentiate properly among reservoirs with good ecological potential from the others (moderate, poor and bad). With that in mind, was tested which one of our zooplankton metrics could differentiate between those levels and discern between the different categories of trophic status. To resolve this, a t-student test was employed. The result of this test indicated that most of our zooplankton metrics were able to differentiate between good or higher from the rest of ecological potentials, while for trophic status levels the proposed metrics varied. Similarly, we used the same test to differentiate between oligotrophy and eutrophy, with results like those obtained for ecological potential. In general, the more sensitive metrics for this study were ZOO, LZOO, CLAD, and ZOO:CHLA.

The results obtained in the present chapter are similar to other studies that previously used zooplankton abundances by functional groups or taxonomic orders within the Mediterranean region (Almeida et al., 2020; García-Chicote et al., 2018; Stamou et al., 2019). Therefore, the proposed metrics using zooplankton abundances and biomasses could be used as a valuable tool for WFD to achieve a correct classification of reservoirs.

Functional groups and machine learning

The studies focused on zooplankton community are vast and diverse, with examples of ecology, ecotoxicology, diversity, habitats, genomic, dispersion, and predation among many others. Conversely, studies on functional groups are scarce (Fernandes et al., 2019). Thus, to try to fill this knowledge gap, in the **fourth and last thesis chapter,** the use of zooplankton functional groups to determine trophic status and ecological potential was investigated. Furthermore, a new approach in zooplankton as a water quality indicator was also implemented, the use of machine learning.

In the previous chapters, zooplankton species richness and dominant genera inside Ebro´s reservoirs were described. To estimate what are the functional groups in the study reservoirs five functional traits were selected: adult body weight, preferred habitat, trophic group, reproduction form and feeding type. With these traits, five major functional groups were identified; filter-feeding copepods, raptorial copepods, cladocerans, microphagous rotifers and raptorial rotifers. In general, functional groups increased in abundance as the trophic state increased, except for raptorial rotifers. The phytoplankton community and morphological variables such as reservoir volume of water and percentage of water in the reservoir were the most important environmental variables directly related in general to functional groups. Other variables such as chlorophyll a and total phosphorus were related to raptorial copepods, while temperature, ammonium, conductivity and alkalinity were more related to filtering copepods and cladocerans.

Several studies have found that the abundance and biomass of raptorial copepods are higher in eutrophic systems (Perbiche-Neves et al., 2021; Pinto-Coelho et al., 2005). Waterbodies with high nutrient levels can present cyanobacterial blooms, these blooms can affect negatively zooplankton community abundances. However, it has been indicated that cyclopoid copepods could not be affected and even increase their densities (Krztoń & Kosiba, 2020). Also, this group has been strongly correlated with the amount of total phosphorus and is considered an indicator of eutrophy (Gyllström et al., 2005; Ochocka, 2021; Pinto-Coelho et al., 2005). Our results indicate also a high relation between raptorial cyclopoid species such as *A. americanus* and total phosphorus. The results from the second, third and this last chapters are in concordance, thus raptorial cyclopoids and their species can be considered as a reliable indicator of low water quality.

On the contrary, in those reservoirs classified as good for their water quality (oligotrophic and good or higher), the dominance, and low abundance, of filtering groups such as calanoids and cladocerans was detected. This has been reported previously in oligotrophic waterbodies being dominated by calanoids and Daphnids (Almeida et al., 2020). Therefore, I would like to highlight the dominance and low density of these groups as an indicator of good water quality environments or low productivity systems.

Since the beginning of scientific observation and the application of scientific method, new advances and technologies have helped to understand our environment. During the last decades, the use of computers and different model has improved science. Nowadays, Machine Learning is a new way to understand data through models that can be very simple to complex and these models can be applied to ecological data (Cutler et al., 2007). Recently, different machine learning techniques have been evaluated in different natural systems, to test which are the best or most suitable for each environment (Humphries et al., 2018). In the case of aquatic systems, it was found that Random Forest technique had the best performance (Visser et al., 2022). Therefore, in our study, through Random Forests, we determined which zooplankton functional groups were more important in determining trophic state and ecological potential.

Our results indicated that raptorial cyclopoid copepods were the most important ZFG, followed by calanoids and cladocerans to determine water quality. Both rotifers functional groups presented minor importance to determine trophic state and ecological potential compared to microcrustaceans. Thus, this indicates that in general, rotifers were the lesser sensitive zooplankton group. This low sensitivity was also noted in the previous chapter since rotifers metrics were less sensitive for differentiation among trophic state and ecological potential classes. On the other hand, in the second chapter I showed that some rotifer species were indicators of eutrophy. In other studies, it has been found that rotifers could also be used as good indicators of trophic state (Duggan et al., 2001; Ejsmont-Karabin, 2012; May & O'Hare, 2005). In the present study, our data and the approaches used, suggest that micro crustaceans would be better elements to determine reservoirs´ water quality. However, the information provided by rotifers indicators species would be a good complement that should not be disregarded.

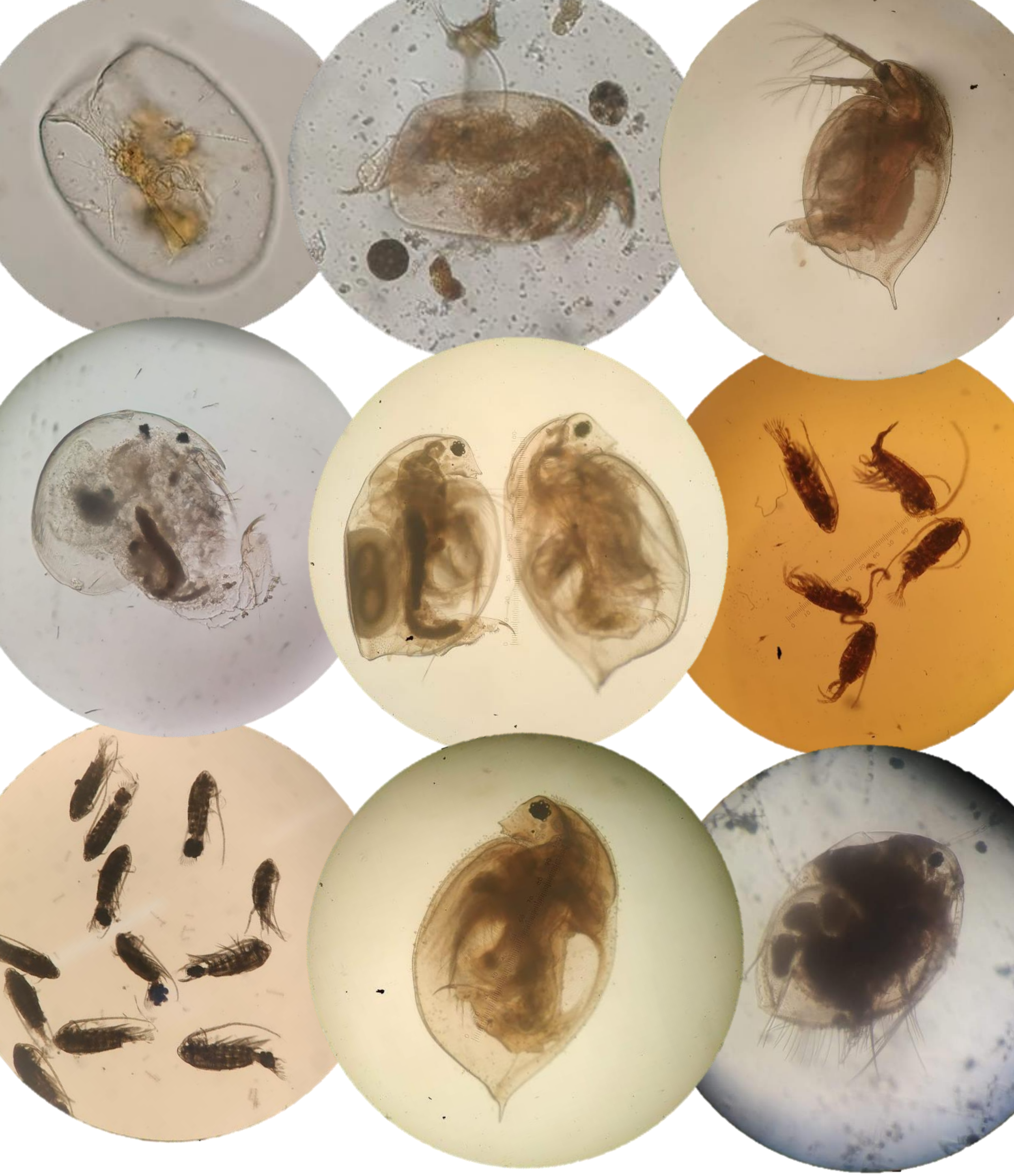
The predictive models that we presented and developed varied in their accuracy from 44% to 77% depending on the ZFG that was used. The best model´s performance to predict trophic state belonged to raptorial copepods, followed by cladocerans and microcrustaceans in general, while to predict ecological potential, the best models were those using data from calanoids followed by those using microcrustaceans. Accuracy models that use ecological data are generally lower compared to other types of predictive models, since, in ecology, many factors or variables cannot be controlled and could exert some type of bias. However, being able to obtain models with high accuracy represents an advance for better water resources management and could be implemented in lakes and reservoirs.

One argument against zooplankton inclusion in the WFD as one biological quality element is that there are no clear thresholds to discern in terms of abundances or biomasses among the different ecological potential levels. Thus, in the fourth chapter, this problem was address by employing classification and regression trees to identify and propose those thresholds. Thresholds were estimated for ecological potential but also for trophic status. So, I propose the following thresholds to differentiate good or higher from moderate status: calanoids <1.4 ind L^{-1}, raptorial cyclopoids <0.38 ind L^{-1} and cladocerans <19.36 ind L^{-1}. While for trophic state to separate between oligotrophic and eutrophic conditions were: calanoids <3.1 ind L^{-1}, raptorial cyclopoids <0.76 ind L^{-1} and cladocerans <19.04 ind L^{-1}.

Similar results were observed in thresholds to differentiate among categories in trophic status and ecological potential in certain zooplankton groups. In the case of the cladocerans abundance threshold to differentiate between oligotrophy from eutrophy and good from moderate was practically the same, near 19 ind L^{-1}. While for copepods such difference was between 1 and 2 ind L^{-1}, which in terms of abundance is a very low margin. Therefore, using the proposed thresholds, the ecological potential and trophic status could be estimated. Since there is a lack of studies proposing a threshold to separate among ecological potential categories, our results could be a good comparison point. Hence, these results should be tested in other reservoirs in different regions and other ecosystems such as lakes to validate or not these thresholds.

In economic terms, inside the WFD requirements for monitoring programs, zooplankton is not included. So, why should we take zooplankton samples? Obtaining zooplankton samples will not cause an impact on the waterbodies monitoring program´s budgets, since zooplankton has a sampling procedure very similar to phytoplankton. So, during sampling campaigns, zooplankton samples can be obtained almost simultaneously with those of phytoplankton. For example, towing two different nets one after the other or in parallel, or filtering water in two different filters in case of use of a limnologic bottle. Also, preservation methods are very similar, fixing with Lugol, alcohol or formalin and samples can be stored in similar glass or plastic vials. Thus, if we are using already the phytoplankton data to determine the ecological potential, we could also use zooplankton valuable information. This could help to perform a better environment characterization and an accurate determination of water quality.

The results from the present thesis indicate that zooplankton community can be used to effectively determine trophic status and ecological potential inside Ebro´s basin reservoirs. This was based on results obtained analyzing zooplankton community data under different approaches; indicator species, abundances and/or biomasses, functional groups and machine learning. Finally, with the data from our research, as conclusion of the present thesis I indicated that zooplankton communities are good indicators to assess water quality and should be included as an element of biological quality within the WFD.



Some zooplankton individuals under stereoscopic microscope.
Scales not available, hence organisms' sizes are different and cannot be compared among them. Valencia University 2018-2022

Final Remarks and Conclusions

Final remarks and conclusions

This doctoral thesis was focused to evaluate zooplankton community as an indicator of water quality. Specifically, to determine trophic status and ecological potential of reservoirs in the Ebro´s basin. This work used zooplankton with different approaches, such as indicator species, metrics based on abundances and biomasses, functional groups and machine learning implementation. The methodology and results in this work should also be tested in other watersheds and aquatic systems such as lakes, to corroborate or discuss the presented results. On the basis of the results achieved in this thesis, the Water Framework Directive should be revised to incorporate zooplankton as a biological quality element.

1. Since the environmental variables that had a higher influence on zooplankton community were chlorophyll a, total phosphorus, phytoplankton abundance, water volume and reservoir size, it means that zooplankton is sensitive enough to detect changes in the environment and may be a good water quality indicator. Hence, zooplankton should be used to determine the trophic status and ecological potential

2. For the very first time the species present at Ullibarri-Gamboa reservoir were reported. The species were the cladocerans *B. longirostris, C. pulchella, D. cucullata, D. longispina*, the Copepod *A. americanus*, the rotifers *A. fissa, B. angularis, C. unicornis, H. oxyuris, K. cochlearis, P. dolichoptera, P. major, S. pectinata* and the zebra mussel *D. polymorpha*. This indicates that there are still many waterbodies where zooplanktonic fauna is unknown. Hence, studies focusing on species presence and diversity in new waterbodies should be continued and supported.

3. The presence of the invasive zebra mussels was detected for the first time at La Sotonera reservoir. So, monitoring waterbodies plays a great importance to detect changes in the community composition that inhabit them.

4. Under the species indicator approach, *A. americanus, D. cucullata, D. brachyurum, B. angulari* and *K. cochlearis tecta* were identified as indicators of eutrophic conditions and moderate-poor ecological potential. On the other hand, *D. longispina*, *A. ovalis* and *A. saltans* were indicator species of oligotrophy and good or higher ecological potential.

5. Zooplankton metrics based on abundances and biomasses were sufficiently sensitive to distinguish among the different trophic status and ecological potential. This indicates that water quality can be determined using the number and biomass of zooplankton individuals present in a reservoir.

6. For the abundance and biomass approach, the best metrics to distinguish between good and bad water quality were total zooplankton, the proportions between total zooplankton and chlorophyll a, large zooplankton, calanoids, cyclopoids, daphnids, and cladocerans in general. Rotifers didn't show a significant difference among water quality categories.

7. Using a functional groups approach, high abundances of raptorial cyclopoid copepods indicate low water quality. While dominance and low abundances of large filter feeders (calanoids and cladocerans) may be used as indicators of good water quality.

8. Under the functional groups and abundances approach, in general, microcrustaceans were better indicators and were more important

to determine water quality than rotifers. Using species as indicators, rotifers were also good indicators for high and low water quality.

9. The implementation of machine learning was a novelty applied in this thesis to zooplankton community as a water quality indicator. Accurate predictive models to determine water quality using functional groups were obtained using Random Forest. Models using microcrustaceans data were more accurate than those using rotifers.

10. Using classification and regression trees, abundance thresholds were established to classify reservoirs' trophic status and ecological potential. Abundance boundaries to separate high and low water quality were similar in the different models. The thresholds were between 0.38 and 0.76 ind L^{-1} for raptorial cyclopoids, calanoids between 1.4 and 4.1 ind L^{-1}, while for cladocerans was 19 ind L^{-1}.

11. Zooplankton data can be very useful to determine water quality, and their use along with phytoplankton data could provide a better understanding and a more accurate trophic status and ecological potential classification. Including zooplankton sampling does not impact monitoring program budgets, since it can be easily obtained in parallel to phytoplankton.

12. The data obtained from this thesis indicate that zooplankton is a good water quality indicator under different approaches. Therefore, zooplankton should be incorporated as one Biological Quality Element within Water Framework Directive.

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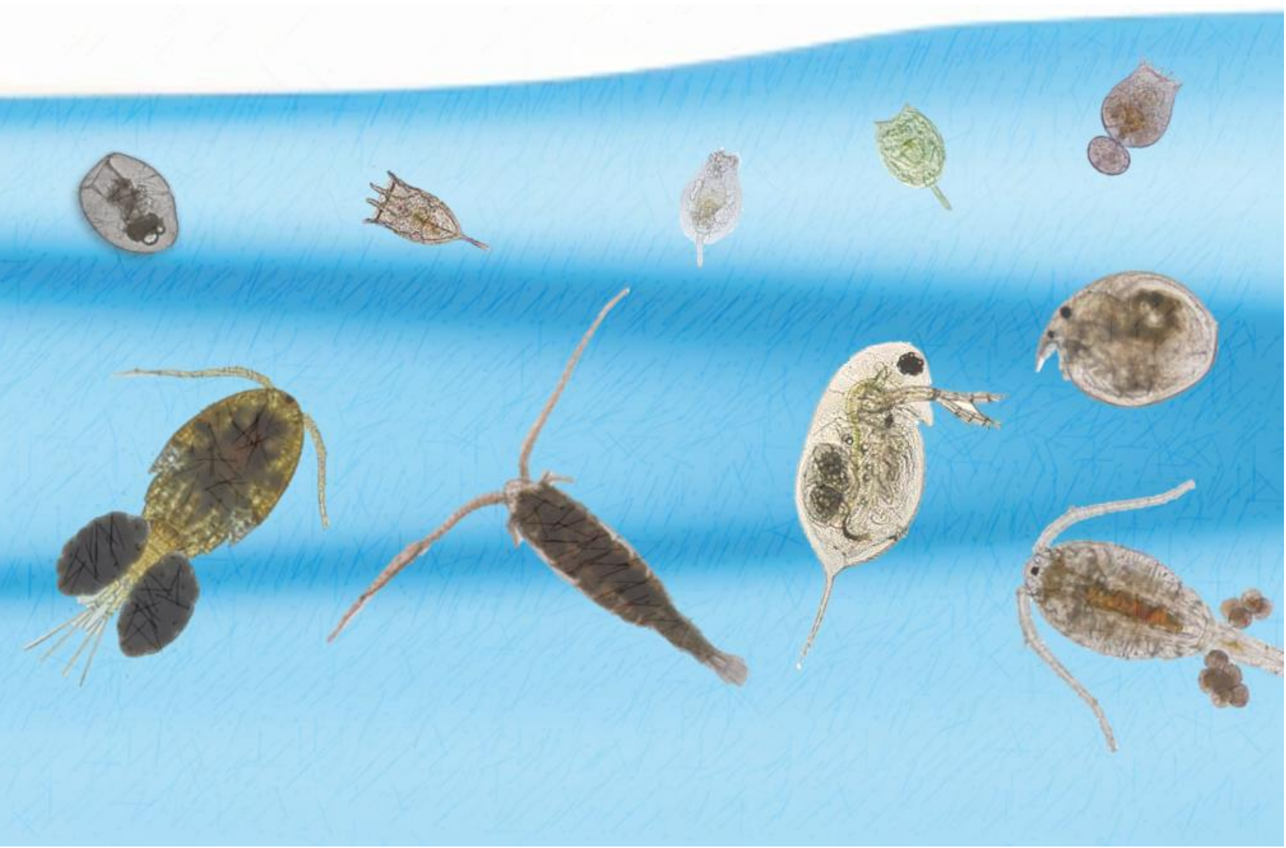

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Chapter 1. Muñoz-Colmenares, M. E., Vicente, E., Soria, J.M, and M. R. Miracle. 2021. **Zooplankton changes at six reservoirs in the Ebro watershed, Spain**. *Limnetica*. 40(2), 279-294. https://doi.org/10.23818/limn.40.19

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Chapter 3. Muñoz-Colmenares, M.E.; Sendra, M.D.; Sòria-Perpinyà, X.; Soria, J.M.; Vicente, E. 2021. **The Use of Zooplankton Metrics to Determine the Trophic Status and Ecological Potential: An Approach in a Large Mediterranean Watershed**. *Water*, 13, 2382. https://doi.org/10.3390/w13172382.

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Chapter 1

Zooplankton changes at six reservoirs in the Ebro watershed, Spain.

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Zooplankton changes at six reservoirs in the Ebro watershed, Spain

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ABSTRACT

Zooplankton changes at six reservoirs in the Ebro watershed, Spain

In the present study, six reservoirs of the Ebro watershed were sampled during summer and autumn of 2016, with the objective of recognizing the zooplankton community, the environmental variables that are correlated with them and update the species checklist. We identify 40 zooplankton species among reservoirs: 21 rotifer species, 10 cladocerans, 8 copepods, and the veliger larvae of the invasive zebra mussel. Species that had higher abundances and biomass were: the rotifer *Polyarthra dolichoptera* (up to 278 ind/L), the cladoceran genera *Daphnia* spp. and *Ceriodaphnia* spp., the copepods *Copidodiaptomus numidicus* (83 ind/L), *Acanthocyclops americanus* (72 ind/L), *Eudiaptomus vulgaris* (62 ind/L) and zebra mussel *Dreissena polymorpha* (540 ind/L). In general, the smaller species were dominant during the summer while the larger species were dominant in the autumn. The density and biomass of zooplankton in four out of six reservoirs during the summer were double that in autumn. The main physicochemical variables correlated with the zooplankton species through a Canonical Correspondences Analysis (CCA) were: chlorophyll a, Secchi disk, total phosphorus, pH and depth. We report for the first time the species presented at the Ullibarri-Gamboa reservoir, and present new species registered in the reservoirs compared with those reported in previous studies. For the first time, the presence of the zebra mussel was detected at La Sotonera reservoir. This indicate its expansion throughout the watershed, suggesting that knowledge of zooplankton species and the changes that occur through time can be a tool for reservoirs and watershed management.

Key words: zooplankton community, reservoirs, Ebro watershed, physicochemical parameters, species seasonal variation

RESUMEN

Cambios en el zooplancton en seis embalses en la cuenca del Ebro, España

En el presente estudio, seis embalses de la cuenca del Ebro fueron muestreados durante las estaciones de verano y otoño de 2016, con los objetivos de conocer la estructura de la comunidad de zooplancton, las variables ambientales con las que se correlación y actualizar los listados de especies. Se identificaron 40 especies de zooplancton entre todos los embalses; 21 especies de rotíferos, 10 de cladóceros, 8 de copépodos y las larvas veligeras del invasivo mejillón cebra. Algunas de las especies que tuvieron mayores abundancias y biomasa fueron: el rotífero Polyarthra dolichoptera *(hasta 278 ind/L), los géneros de cladóceros* Daphnia *spp, y* Ceriodaphnia *spp., los copépodos* Copidodiaptomus numidicus *(83 ind/L),* Acanthocyclops americanus *(72 ind/L),* Eudiaptomus vulgaris *(62 ind/L) y el mejillón cebra* Dreissena polymorpha *(540 ind/L). En general, durante el verano las especies de menor tamaño fueron las dominantes mientras que en el otoño tuvieron una mayor dominancia las especies de mayor tamaño. La densidad y biomasas del zooplancton en cuatro de los seis embalses durante el verano fue el doble que en otoño. Las principales variables fisicoquímicas correlacionadas a las especies de zooplancton a través de un Análisis de Correspondencias Canónicas (ACC) fueron: clorofila a, disco de Secchi, fosforo total, pH y la profundidad. Se reportan por primera vez las especies presentes en el embalse de Ullibarri-Gamboa, además de que se presentan nuevas especies registradas en el resto de los embalses contra aquellas reportadas en estudios anteriores. Se detecto por primera vez la presencia del mejillón cebra en el embalse de La Sotonera, indicando su expansión a través de la cuenca, esto nos sugiere que el conocimiento de las especies del zooplancton y los cambios que presentan a través del tiempo puede ser una herramienta para el manejo de los embalses y la cuenca.*

Palabras clave: *comunidad del zooplancton, embalses, cuenca del Ebro, parámetros fisicoquímicos, variación estacional de especies*

INTRODUCTION

Zooplankton is, an important component inside the freshwater ecosystem, playings a big role in the transfer of energy in the aquatic food web between primary producers and higher consumers, while significantly contributing to the recycling of nutrients (Lampert & Sommer, 1997).

Besides their essential role in trophic levels of aquatic environments, this group can also provide valuable information that other groups cannot. For example, changes in certain metrics such as, size, proportion of large and small zooplankton, mean of body weight and proportion of resting eggs together with the zooplankton:phytoplankton biomass ratio, which can indicate a "top-down" process (Jeppesen *et al.*, 2011). Top-down control is one of the main attributes of zooplankton. This occurs when zooplankton consumes high quantities of phytoplankton and becomes a pressure factor, this pressure can determine the composition of phytoplankton assemblage and decrease their abundances and biomass (Naselli-Flores & Rossetti, 2010). On the other hand, the zooplankton community can respond quickly to phytoplankton blooms during the bottom-up control (Carpenter *et al.*, 1985), such as, changes in the biomass, the proportion of calanoids copepods and numbers of rotifers could indicate this process (Jeppesen *et al.*, 2011). Due to their pivotal position in the transfer of nutrients and energy in aquatic food webs and the valuable data they can provide, it is essential to have a wide knowledge of zooplankton composition and the factors related to this group (Caroni & Irvine, 2010).

All the species and individuals that make up the zooplankton community exhibit diverse responses to changes (Stemberger *et al.*, 2001). These changes can be done by biotic (e.g. food availability, predation and competition) and abiotic (physical and chemical habitat conditions: temperature, dissolved oxygen, pH, etc.) factors, as both can affect the species richness, increasing or decreasing their abundances and biomass, and promoting shifts in their diversity (Jeppesen *et al.*, 2000; Wetzel, 2001; Dodson *et al.*, 2009; Bonecker *et al.*, 2013). Hence, studies focused on such factors can provide useful information to manage natural resources (Gulati *et al.*, 1990) as well as the understanding of how its community structure (species richness, density and abundances) varies with time and in different aquatic systems (Dodson *et al.*, 2009; Boix *et al.*, 2008).

Seasonal variation also has an important role in waterbodies. Over the course of a year many environmental variables can suffer big changes depending on the season (Margalef, 1983). On a regional and local scale, these seasonal changes in natural components, in addition to anthropogenic pollution, can impact on aquatic communities and affect the zooplankton groups in different ways (Tavernini *et al.*, 2009).

Many studies have correlated density, species richness and the presence or absence of zooplankton, for example, rotifers (Sladečeck, 1983; Ejsmont-Karabin, 1995, 2012; May & O'Hare, 2005) and micro-crustaceans (Pinto-Coelho *et al.*, 2005) to the trophic gradient. Moreover, zooplankton, can be an element in evaluating the trophic state of reservoirs and lakes (Haberman *et al.*, 2007; Haberman & Haldna, 2014) and a good indicator of the different trophic states related to natural processes, man-made activities and climate changes (Jeppesen *et al.*, 2011). Recently, in man-made reservoirs have proved that even zooplankton density can be a tool to determinate the trophic state of a large watershed in Spain (Garcia-Chicote *et al.*, 2018). Although the Water Framework Directive has the aim of evaluating the European waters through several Biological Quality Indicators, zooplankton and its valuable data is not included as one of these indicators.

Despite the Ebro watershed being the second large watershed in Spain, studies related to zooplankton presence in the reservoirs are few and focus principally on rotifera phylum´s description or distribution (De Manuel & Armengol, 1993; De Manuel, 2000). In the present study we focus on zooplankton communities of six reservoirs located across the watershed. These were chosen due to the fact that existing data is more than 30 years old (De Manuel & Jaumel, 1993) or no previous data was available. The lack of information of these reservoirs throughout the last three decades could significate changes in species composition and non-detected invaders. Therefore, it´s important to update the current knowledge on zooplanktonic fauna in this watershed and know how environmental variables can affect the composition of communities through seasons. Thus, all this information could be a helpful tool for reservoirs management.

The main objectives of this study were; first, report and compare the zooplankton composition (species richness, density and biomass) during two studied seasons (summer and autumn) in six reservoirs at the Ebro watershed. Second, determine the environmental variables related to the zooplankton groups structure (density and biomass). Third, update information on the zooplanktonic fauna and verify if new species are present in the reservoirs compared with available data of previous studies.

MATERIAL AND METHODS

Study area

The data presented in this study was obtained from six reservoirs, located in different areas and altitudes along the Ebro watershed (Fig. 1). Each reservoir was sampled at the beginning of two different seasons in 2016: summer (last week of June) and autumn (last week of September). One sampling point was established at each reservoir in the deepest part of the reservoir at 300-500 meters from the dam.

Environmental Variables

For each reservoir the following variables were measured along the water vertical profile, temperature, conductivity, dissolved oxygen, pH, turbidity and chlorophyll *a*, all *in situ* measurements, by

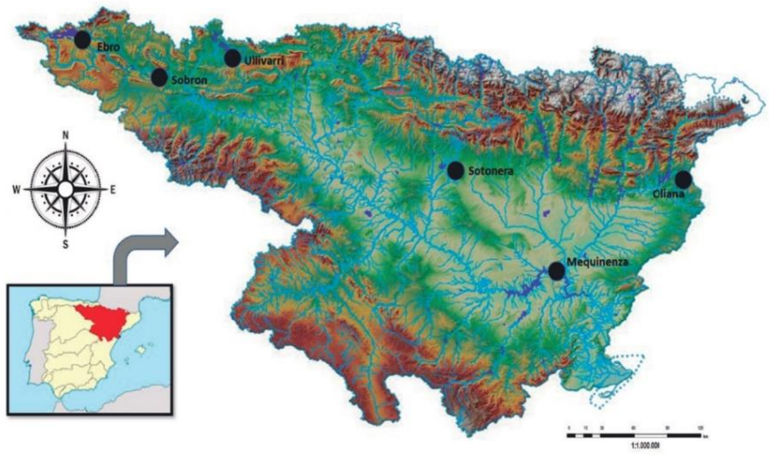

Figure 1. Location of the Ebro Watershed with the sampled reservoirs. *Localización de la Cuenca del Ebro con los embalses muestreados.*

means of a multiparametric devise Sea-Bird 19 plus V2. The depth of the photic zone was calculated by measuring the light penetration using a quantometer. The water transparency was determined measuring the Secchi disk depth (SD). An integrative water sample was collected from the photic zone of each reservoir using a 25 mm ballasted tube technique for *ex-situ* analyses (Vicente *et al.*, 2005). For measurements of the following variables, we used the standard methodology (APHA, 1998) described for suspended solids (APHA 2540D), turbidity (ISO7027-1999), total nitrogen (TN) (APHA method 4500-N C), total phosphorus (TP) (4500-P B/APHA 4500-P C), and chlorophyll *a* (Shoaf & Lium, 1976). The complete data set of environmental variables can be found at C.H.E. (2016).

To estimate the reservoir´s trophic conditions, we used the criteria of the trophic state index (TSI) (Carlson, 1977). The TSI´ values of each reservoir were obtained with the following formulae (Carlson & Simpson, 1996):

Total phosphorus; $TSI(TP) = 14.42 \ln(TP) + 4.15$
Chlorophyll *a*, $TSI(Chl\text{-}a) = 9.81 \ln(Chl\text{-}a) + 30.6$
Secchi disk, $TSI(SD) = 60 - 14.41 \ln(SD)$

Total phosphorus and Chl-*a* are measured in micrograms per liter (μg/L) and Secchi disk depth is expressed in meters. TSI is the average value of the three above mentioned variables.

$$TSI' = [(TSI(TP) + TSI(Chl\text{-}a) + TSI(SD))/3]$$

Zooplankton samples

The zooplankton samples were collected using a Ruttner bottle with a capacity of 2.7 L. For each reservoir were taken two Ruttner bottles to obtain 5.4 liters of water sample, then the sample was filtered through 30 μm mesh size Nytal, fixed with formaldehyde at 4 % final concentration and stored in a hermetic glass vial. The sample depth was established in each reservoir at the beginning of oxygen decline, where has been reported as the richest zone of zooplankton fauna (Miracle & Vicente, 1983). Also, a zooplankton vertical tow net of 50 μm mesh size Nytal was towed from 30 m deep to the surface, collected and fixed with formalin. These vertical tow net samples were taken mainly for taxonomic purposes.

Zooplankton species were identified using the following guides: Ruttner-Kolisko (1974), Koste (1978), Nogrady *et al.*, (1995) and Nogrady & Segers (2002) for rotifers, Alonso (1996) for cladocerans, and Dussart (1967, 1969) for copepods.

For quantitative results, we used the samples taken from the Ruttner bottles, all individuals were counted using a Sedgewick Rafter-type counting chamber under inverted microscopy. After individuals were counted and densities were obtained, we calculate the biomass, to determine it, a minimum of 30 specimens of all species were measured and using the formulas that relate the total length with the dry weight of the specimens were obtained the corresponding conversion factors (Ruttner-Kolisko, 1977; Dumont *et al.*, 1975; Culver *et al.*, 1985). The Shannon–Wiener diversity index (H') (Shannon & Weaver, 1963) was calculated from data on the abundance of zooplankton for each reservoir at both seasons.

Statistical analysis

The correlation coefficients between zooplankton data and the environmental factors were calculated by linear Pearson correlations. Analysis of similarity (ANOSIM) tests were performed on the zooplankton data to determine which, if any, reservoirs showed significant differences in zooplankton community structure between the two seasons. ANOSIM is a nonparametric analogue to analysis of variance and tests for multivariate differences between groups based on Bray-Curtis distance and rank dissimilarity. Also, we ran a similarity percentage routine (SIMPER), to test which zooplankton species were contributing to the community changes. The SIMPER routine uses average Bray-Curtis dissimilarities between all pairs of sites to produce a percent contribution from each species, identifying the species most responsible for the dissimilarity (Clarke & Warwick, 2001).

To determine the influence of different factors on zooplankton we performed two canonical correspondence analysis (CCA). For the first, we

Table 1. Complete data of physicochemical variable measurements during both seasons, data modified from C.H.E (2016). *Datos completos de los parámetros fisicoquímicos medidos durante las dos sesiones. Datos modificados de C.H.E. (2016).*

		Summer						Autumn					
Parameter	**unit**	ULL	MEQ	EBR	OLI	SOB	SOT	ULL	MEQ	EBR	OLI	SOB	SOT
Temperature	**°C**	20.80	23.62	18.15	18.87	21.59	23.50	19.49	24.64	18.41	22.99	17.77	23.29
Disolved oxygen	**mg/L**	7.16	6.79	7.84	8.70	7.31	8.04	5.85	3.52	7.95	7.45	6.87	7.29
Conductivity	**µS/cm**	244	815	188	187	324	331	219	1288	195	270	255	318
pH		8.43	8.24	8.18	8.45	8.31	8.34	8.09	7.94	7.87	8.35	7.89	8.38
Depth	**m**	23	57	20	50	27	20	21	53	14	37	27	12
Secchi	**m**	3.50	3.70	4.50	2.90	1.70	2.25	7.75	3.80	1.40	2.70	2.00	1.10
Suspended solids	**mg/L**	1.12	2.13	1.29	4.32	4.47	3.40	0.79	2.02	7.41	4.11	3.39	7.56
Turbidity	**NTU**	1.88	1.88	2.89	3.80	4.37	1.61	1.55	1.22	6.99	3.86	4.47	4.74
Alkalinity	**Meq/L**	2.28	3.12	1.24	1.52	2.32	2.52	2.00	2.85	1.24	1.92	1.88	2.24
Chl-*a*	**µg/L**	2.26	3.66	2.26	6.73	11.13	3.38	1.37	3.69	5.00	21.14	3.03	3.69
TN	**µg/L**	660	217	450	680	810	450	324	1692	427	622	484	276
TP	**µg/L**	9.41	9.12	13.54	80.78	25.50	8.39	22.44	13.00	26.48	25.00	19.29	16.00

analyzed those variables that are corresponded to the principal zooplankton species. For the second, we performed an analysis using the zooplankton groups (rotifers, copepods, cladocerans and zebra mussels). For each CCA we included the densities and or biomass of zooplankton and the following environmental variables (temperature, dissolved oxygen, conductivity, turbidity, pH, Secchi disk, depth, nutrients (TP and TN) and Chl-*a*. In order to normalize the data, they were transformed logarithmically $\text{Log}(x + 1)$, except for pH. The models were tested using Monte Carlo permutation ($n = 499$). Nauplius, copepodites and bdelloid rotifers were excluded since they were not identified to species level. Both CCAs were executed using the Canoco 4.5 for Windows computer program (Ter Braak & Šmilauer, 2002).

RESULTS

Environmental parameters

During the two seasons of this study the physical and chemical parameters varied at the different reservoirs, complete data is reported in Table 1. The water temperature on average was higher during summer in all waterbodies, except at Oliana, where it was higher in autumn. In general, the dissolved oxygen presents higher values during summer than those in autumn. The pH values do not show an important difference between seasons because the buffer effect of the bicarbonate in the waters and the conductivity values were stable (with exception of Mequinenza during autumn with a peak of 1288 µS/cm). Suspended solids in both seasons were similar in four of the six reservoirs, however, data from the Ebro and La Sotonera reservoirs during the autumn were double compared to the summer data. The Secchi disk visibility presented a wide variability among reservoirs and seasons: Ebro, Oliana and La Sotonera had higher values in summer, nevertheless, Ullibarri-Gamboa, Mequinenza and Sobrón were higher during autumn.

In the case of Chl-*a*, higher values were presented during the summer at Ullibarri-Gamboa and Sobrón, and during the autumn at Ebro and Oliana. The Oliana reservoir (autumn) had the biggest Chl-*a* concentration of all the study (21.14 µg/L). Finally, Mequinenza and La Sotonera had similar values during both seasons (average of 3.3 µg/L and 3.5 µg/L respectively). Total Nitrogen (TN) values at 5 of the reservoirs were higher during summer, only Mequinenza presented a high peak in autumn (1692 µg/L). The higher values of total phosphorus (TP) were reported in autumn, except at Oliana (80.78 µg/L) and Sobrón (25.5 µg/L), where the higher values were during summer.

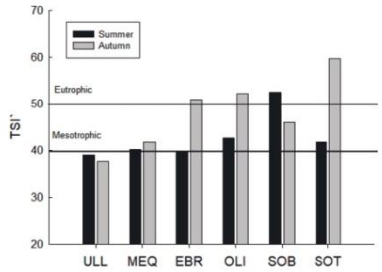

Figure 2. Reservoir TSI´ values, black charts (summer) and gray charts (autumn). *Valores de TSI´ de los embalses, barras en negro (verano) y gris (otoño).*

Trophic state

With the data obtained, we calculated the Trophic State Index (TSI) for every reservoir and season, the waterbodies were ordinated according to their TSI from lower to higher values. Ullibarri-Gamboa shows the lower trophic value in both seasons (39.01, summer and 37.70, autumn) and is classifiedd as oligotrophic. While Sobrón had the higher value during the summer (52.5) and La Sotonera in autumn (59.8). The reservoirs during the summer generally presented values between 30 to 50 and during autumn the values increase from 40 to 60. According to Carlson (1996), most reservoirs are classified as mesotrophic, however, Ebro, Oliana and La Sotonera are eutrophic during the autumn and Sobrón during the summer (Fig. 2).

Zooplankton

We registered a total of 40 zooplankton species during both seasons in the six reservoirs (Table 2). The rotifers were the group with more species reported (21), followed by cladocerans (10) and copepods (8). Since the veliger larvae of the zebra mussel invader (*Dreissena polymorpha*) were found at 4 reservoirs, they were considered a separate group inside this study and both abundance and biomass were counted. La Sotonera presented the highest number of species with, 13 in each season, followed by Sobrón with 12 in the summer, Oliana and Mequinenza with 11 during summer. This same species richness was present in Ebro and Ullibarri-Gamboa in the autumn. The lowest number of species was in Sobrón with only 6 during the autumn (Table 2). On average, each reservoir presented 10 zooplankton species per season. The rotifer *Polyarthra dolichoptera* was presented on all reservoirs in at least one season, followed by the zebra mussel, which was detected on four reservoirs during both seasons. The two copepod species *Cyclops vicinus* and *Cyclops* sp., and some rotifers were only presented in one reservoir during one season (Table 2).

The only previous study on these reservoirs was performed during 1987-1988 and reports data of summer and winter seasons. However, to compare species composition per season we only used the summer data from both studies, since the other season is not the same and cannot be compared equally (winter from the previous study and fall in the current). To indicate new registers for each reservoir we verified that species were not present in the data of both seasons from the previous study. The complete list of species present of the previous study can be found in De Manuel & Jaume (1993).

The new registers of zooplankton species for each reservoir are: Sotonera (*Bosmina longirostris*, *Ceriodapnia dubia*, *Ceriodaphnia pulchella*, *Daphnia galeata*, *Diaphanosoma mongolianum*, *Acanthocyclops americanus*, *Anuraeopsis fissa*, *Polyarthra major*, *Ascomorpha ecaudis* and *D. polymorpha*). Ebro (*Eudiaptomus vulgaris*, *B. longirostris*, *D. mongolianum*, *Conochilus unicornis*, *Trichotria tetractis*, *P. major*, *Tricochercca cylindrica*). Mequinenza (*Copidodiaptomus numidicus*, *Thermocyclops dybowskii*, *A. ecaudis*). Sobron (*Daphnia cucullata*, *Cyclops vicinus*, *Asplanchna priodonta*, *P. major*, *A. fissa*) and Oliana (*A. americanus*, *E. vulgaris*, *C. sphaericus*, *D. mongolianum*, *Kellicotia longispina*). Since there is not previous data available for Ullibarri-Gamboa reservoir, all 14 species reported for this study are first register (Table 2).

Table 2. Complete list of zooplankton species found in the six reservoirs. *Listado completo de las especies de zooplancton presentes en los seis embalses.*

	Summer						Autumn					
	ULL	MEQ	EBR	OLI	SOB	SOT	ULL	MEQ	EBR	OLI	SOB	SOT
Cladocera												
Bosmina longirostris	X			X	X	X	X		X	X		X
Ceriodapnia dubia		X		X				X				
Ceriodaphnia pulchella					X	X	X		X	X	X	X
Chydorus sphaericus					X				X	X		
Daphnia cucullata	X				X		X				X	
Daphnia galeata				X		X				X		
Daphnia longispina							X		X			
Daphnia pulicaria			X									
Diaphanosoma mongolianum		X				X		X		X		
Pleuroxus sp.										X		
Copepoda												
Acanthocyclops americanus				X		X	X			X		X
Copidodiaptomus numidicus		X						X				
Cyclops abyssorum			X						X			
Cyclops vicinus					X							
Eudiaptomus vulgaris			X	X					X	X		
Neolovenula alluaudi						X						X
Thermocyclops dybowskii		X						X				
Cyclops sp.											X	
Rotifera												
Anuraeopsis fissa	X										X	X
Ascomorpha ecaudis		X				X		X				
Asplanchna priodonta		X		X		X						
Brachionus angularis	X											
Brachionus calcyflorus					X							
Brachionus havanaensis											X	
Brachionus quadridentatus					X							
Conochilus unicornis	X		X									
Filinia longiseta									X			
Hexarthra fennica								X				
Hexarthra oxyuris							X					
Kellicotia longispina longispina				X								
Keratella cochlearis	X	X	X	X		X	X	X				X
Keratella cochlearis tecta						X	X					X
Keratella quadrata				X								
Polyarthra dolichoptera	X	X	X	X	X	X	X	X	X	X		
Polyarthra major					X		X		X			X
Synchaeta pectinata	X				X	X						X
Synchaeta sp.			X							X		
Trichocerca cylindrica									X			
Trichotria tetractis			X									
Others												
Dreissena polymorpha	X	X			X	X	X	X			X	X

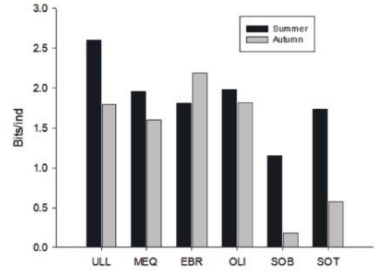

Figure 3. Shannon-Wiener diversity index (black bars represents summer, gray bars autumn). *Diversidad de Shannon-Wiener (barras negras representan verano, grises otoño).*

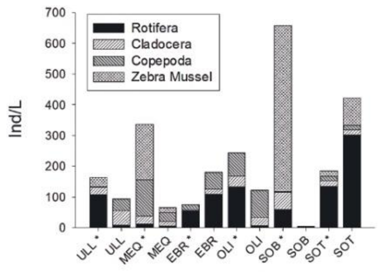

Figure 4. Abundances of zooplankton groups in the six reservoirs, (*) indicate summer values. *Abundancias de los grupos de zooplancton en los seis embalses, (*) indica valores de verano.*

Density and biomass

The zooplankton density varied in each reservoir and season, the average of individuals for all the reservoirs during summer was 277 ind/L, that was higher than in autumn with 148 ind/L. The higher densities in most of reservoir belong to rotifers and zebra mussels, except in Ullibarri-Gamboa, Mequinenza and Oliana during winter (Fig. 4).

In terms of biomass, microcrustaceans have a bigger role instead of rotifers, and each reservoir varied in quantity and group that dominates during both seasons. The reservoirs with major changes between biomass were La Sotonera (dominated during the summer for microcrustaceans to zebra mussels in fall), Sobrón (zebra mussels to cladocerans) and Ullivarri-Gamboa (cladocerans, rotifers and zebra mussels to microcrustaceans mainly) (Table 3). The Shannon-Wiener index indicated that diversity in the Ullibarri-Gamboa reservoir was the highest overall 2.59 bits/ind in summer. The lowest diversity was found in Sobrón during autumn with only 0.18 bits/ind (Fig. 3).

Data analysis

Through the linear Pearson correlations between environmental factors and zooplankton groups we found that pH was significantly correlated with the density of rotifer group (r^2 0.35, $p < 0.05$). Also, both zebra mussel density and biomass were positively correlated with Chl-*a* (r^2 0.60, $p < 0.05$ and r^2 0.62, $p < 0.05$ respectively). Besides, copepods density (r^2 0.39, $p < 0.05$) and biomass (r^2 0.34, $p < 0.05$) were correlated with the reservoir´s depth. Other correlations were not significant ($p > 0.05$). The Analysis of similarity (ANOSIM) doesn't show any difference between both seasons ($p > 0.05$). The contribution of the individual taxa in the dissimilarity of zooplankton was low (SIMPER values < 5 %), being *A. priodonta*, *C. pulchella*, *Synchaeta pectinata* and *D. mongolianum* the responsible for the cumulative of 20 % in the variance of dissimilarity between seasons.

The first CCA, related the physicochemical variables with the principal zooplankton species. The first two axes explains 45.2 % of the variance (*p* value 0.001 in the Monte Carlo permutation test). Temperature, conductivity and depth are strongly related to copepods (*C. numidicus*, *Cyclops* sp. and *T. dybowskii*) and the cladoceran *C. dubia*. Two of the most abundant rotifers are related with the pH (*S. pectinata* and *C. unicornis*) in addition to the cladoceran *D. cucullata*. A big group composed principally by cladocerans, few copepods and rotifers were related to dissolved oxygen (DO), turbidity, TP, Chl-*a* and suspended solids (SS) (Fig. 5). The rotifer *P. dolichoptera*

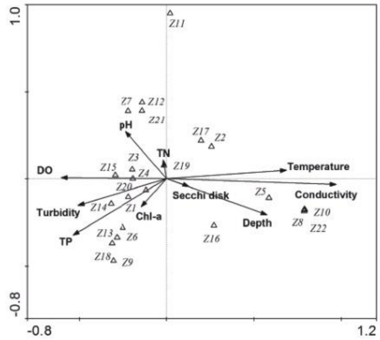

Figure 5. Canonical Correspondences Analysis of the 22 zooplankton main species. DO = Dissolved oxygen, TP = Total phosphorus, TN = Total nitrogen, Chl-*a* = chlorophyll *a*. *Análisis de Correspondencias Canónicas de las 22 especies principales del zooplancton, DO = Oxígeno disuelto, TP = Fosforo total, TN = Nitrogeno total, Chl-a = clorofila.* Z1 *Acanthocyclops americanus*, Z2 *Asplanchna priodonta*, Z3 *Bosmina longirostris*, Z4 *Ceriodaphnia pulchella*, Z5 *Ceriodapnia dubia*, Z6 *Chydorus sphaericus*, Z7 *Conochilus unicornis*, Z8 *Copidodiaptomus numidicus*, Z9 *Cyclops abyssorum*, Z10 *Cyclops* sp., Z11 *Cyclops vicinus*, Z12 *Daphnia cucullata*, Z13 *Daphnia galeata*, Z14 *Daphnia longispina*, Z15 *Daphnia pulicaria*, Z16 *Diaphanosoma mongolianum*, Z17 *Dreissena polymorpha*, Z18 *Eudiaptomus vulgaris*, Z19 *Neolovenula alluaudi*, Z20 *Polyarthra dolichoptera*, Z21 *Synchaeta pectinata*, Z22 *Thermocyclops dybowskii*.

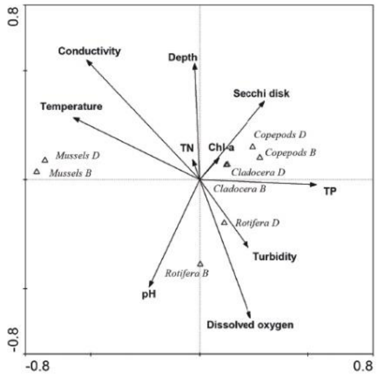

Figure 6. Canonical Correspondences Analysis of the zooplankton groups, D = density, B = biomass, TP = Total phosphorus, TN = Total nitrogen, Chl-*a* = chlorophyll *a*. *Análisis de Correspondencias Canónicas de los grupos del zooplancton, D = densidad, B = biomasa, TP = Fosforo total, TN = Nitrogeno total, Chl-a = clorofila.*

was in the middle of the ordination plot, this rotifer was present in all reservoirs during both seasons, their highest abundances were during the summer at La Sotonera (279 ind/L) and Oliana (125 ind/L). The *Daphnia* group was related to DO, TP and turbidity. The *Daphnia* species were present in five reservoirs and their seasonality was split into those which had higher abundances in summer (*D. cucullata*, *D. galeata* and *D. pulicaria*) and in autumn (*Daphnia longispina*). Finally, the copepod *C. vicinus* is not related to any variable and the zebra mussels are slightly connected with pH and alkalinity (Fig. 5).

In the second CCA, we analyzed the environmental variables related with the zooplankton density and biomass, the first two axes represent the most explanatory value (93.2 %) (p value > 0.05 in the Monte Carlo permutation test). This CCA indicates that in the first axis the trophic indicators are related (SD, Chl-*a* and TP). These principal indicators are related with both cladocera and copepoda density and biomass, while rotifer density is related to combination of TP, turbidity and DO. The biomass of rotifera group presents a similar relation with pH instead of turbidity. Finally, both density and biomass of zebra mussels are related to temperature and conductivity (Fig. 6).

DISCUSSION

Sommer *et al.* (1986) together with the PEG (Plankton Ecology Group) proposed a model where sequential statements describe the changes in zooplankton and phytoplankton communities in lakes. In these statements, they described that in summer the smaller groups with short generational life cycle dominate and during autumn large species appear. In our study, we found that most of the changes in reservoirs' communities followed these statements, for example, the general tendency at Oliana was that rotifers had high abundances during summer, then, during autumn this group tended to decrease and microcrustaceans increased in number becoming the dominant group. Ullibarri-Gamboa presented a similar tendency but with higher abundances of rotifers and mussels during the summer, followed by the increase of copepods and cladocerans during autumn. At the Ebro and La Sotonera reservoirs, the number of all groups increase in autumn but with the rotifers being the dominant group. However, at Mequinenza the zebra mussels and copepods were dominants during summer reaching up 350 ind/L, but with a decrease during autumn. Sobrón shows a similar tendency, the summer was dominated by the zebra mussel (540 ind/L) and in the next period densities of all groups decreased dramatically. For these two last reservoirs several factors could explain these changes, such as an extreme fish predation (Amundsen *et al.*, 2009; Ginter *et al.*, 2019), the establishment of the sessile stage of mussels in any surface decreasing the number of the planktonic larvae (Claudi & Mackie, 1994) or even some criteria that were not taken in count in the previous model, such as the food quality and the trophic level of each reservoir (Sommer *et al.*, 2012).

Biodiversity is strongly related with environmental factors (Jeppesen *et al.*, 2000), while some physiochemical parameters such as temperature, dissolved oxygen, pH, etc., can have positive or negative effects on zooplankton (Wetzel, 2001). One of the more efficient analyses to correlate the zooplankton communities with the physical and chemical variables is the CCA (Attayde & Bozelli, 1998). Data from our CCA analysis shows the rotifer *P. dolichoptera*, which was positioned in the middle of the ordination plot, due to their high tolerance to different environments conditions (Bērziņš & Pejler, 1989), nowadays it has a wide distribution in many water bodies around the world (Segers, 2007). The copepod *Neolovenula alluaudi*, that is typically from the Mediterranean area (Miracle, 1982), also was positioned near the center of the CCA. We can infer that they possess high tolerance, however, compared to the previously mentioned rotifer, it was only present at La Sotonera reservoir. The populations of this copepod are moving from the south and are now found in several water bodies along the Iberian Peninsula (Alfonso & Belmonte, 2013; Miracle, 1982). Thus, this copepod was reported at Mequinenza 30 years ago (De Manuel & Jaume, 1993), but not found during the present study. Furthermore, at the Mequinenza reservoir the presence of silurids is well documented and the early stages of this fish can consume copepods and large cladocerans individuals as the *Daphnia* species, they can promote the small-size species such as *C. dubia* and *D. mongolium*, (Miranda *et al.*, 2010). Also, in this reservoir no *Daphnia* species were recorded, probably due the combination of predation and lower levels of oxygen compared to other reservoirs (Hanazato, 1996).

The copepods *C. numidicus* and *T. dybowskii* were correlated with conductivity, temperature and depth. It is well known that big-sized zooplankton species perform a daily vertical migration to avoid depredation (Hays, 2003; Lampert, 1989). The study of Caramujo & Boavida (2000) found that these two copepod species can be consumed in large numbers by fishes, for this reason, their populations are settled in deepest water bodies. In this study, we found both species only at Mequinenza, which has an average 50 m of depth in both seasons. The biggest copepod found in this study was *C. numidicus* and it provides a high percentage of total biomass and density of all copepods, thus, Pearson correlation was significative in terms of depth for this group.

In the CCA for groups (Fig. 6) the rotifer biomass was also correlated to pH, other studies have shown that this parameter can affect the rotifer occurrence (Bērziņš, 1987) and their assemblage in reservoirs (Devetter, 1998).

The complex of abundances and biomasses of microcrustaceans (copepods and cladocerans), were related with the components that conform the trophic state since they are influenced by the Secchi disk, TP and Chl-*a*. Some authors have indicated that large species of these groups can be used as an indicator of oligotrophic state (Pejler, 1983; Moss *et al.*, 2003; Kane *et al.*, 2009; Haberman & Maldna, 2014). Usually, at higher trophic level, large species are replaced by small species (Lampert & Sommer, 1997). The Ebro, Oliana and La Sotonera reservoirs, during the autumn, were mesotrophic to eutrophic, and densities of larger species as *Daphnia* spp. decay while smaller cladocerans such as *B. longirostris* and *Ceriodaphnia* spp. increase.

Despite the limitations of this work (and taking in count the low number of reservoirs sampled compared with the watershed size), with the data obtained from the CCA we could hypoth-

Table 3. Density (ind/L), Biomass (mg/L) and their percentage (%) of zooplankton groups present on the six reservoirs. *Densidad (ind/L), Biomasa (mg/L) y el porcentaje (%) de los grupos del zooplancton presentes en los seis embalses.*

	Summer				Autumn			
	ind/L	mg/L	ind/L %	mg/L %	ind/L	mg/L	ind/L %	mg/L %
Ullibarri-Gamboa								
Cladocerans	23.46	31.15	14.4	47.14	47.12	95.06	49.8	73.95
Copepods	2.31	0.34	1.42	0.52	38.85	32.87	41.06	25.57
Rotifers	107.69	22.23	66.12	33.64	8.27	0.47	8.74	0.36
Mussels larvae	29.42	12.35	18.06	18.7	0.38	0.16	0.41	0.13
Total	162.88	66.07	100	100	94.62	128.55	100	100
Mequinenza								
Cladocerans	24.62	47	7.31	11.3	15	29.54	22.61	36.75
Copepods	118.08	287.74	35.05	69.19	29.81	43.88	44.93	54.59
Rotifers	13.08	5.03	3.88	1.21	5.77	0.33	8.7	0.41
Mussels larvae	181.15	76.08	53.77	18.3	15.77	6.62	23.77	8.24
Total	336.92	415.86	100	100	66.35	80.37	100	100
Ebro								
Cladocerans	3.85	14.62	5.12	23.46	16.73	29.96	9.25	23.29
Copepods	15.96	42.38	21.23	68.04	55.19	91.83	30.5	71.39
Rotifers	55.38	5.29	73.66	8.5	109.04	6.83	60.26	5.31
Total	75.19	62.29	100	100	180.96	128.62	100	100
Oliana								
Cladocerans	34.8	75.9	14.22	44.69	24.81	36.71	20.31	48.05
Copepods	76.92	86.86	31.42	51.14	89.42	39.29	73.23	51.43
Rotifers	133.08	7.09	54.36	4.17	7.88	0.4	6.46	0.52
Total	244.8	169.85	100	100	122.11	76.4	100	100
Sobrón								
Cladocerans	54.23	104.17	8.24	29.75	0.77	1.62	19.05	64.52
Copepods	3.08	3	0.47	0.86	1.73	0.35	42.87	13.83
Rotifers	60.58	16.21	9.21	4.63	0.38	0.06	9.53	2.32
Mussels larvae	540	226.8	82.08	64.77	1.15	0.48	28.55	19.34
Total	657.88	350.19	100	100	4.04	2.5	100	100
Sotonera								
Cladocerans	16.92	24.19	9.19	39.5	15.77	24.15	3.74	23.44
Copepods	16.73	20.68	9.08	33.77	15.58	25.92	3.7	25.16
Rotifers	134.62	9.67	73.07	15.78	302.5	16.29	71.83	15.81
Mussels larvae	15.96	6.7	8.66	10.95	87.3	36.67	20.73	35.59
Total	184.23	61.24	100	100	421.15	103.03	100	100

esize the zooplankton groups, such as, copepods and cladocerans could be affected firstly and their structure modified if the variables that are more related or affect these groups change for several factors, such as, climate change, new invasive species and or anthropogenic impacts.

The zooplankton community normally varied through months, seasons or years, and the species replacement can happen quickly or change gradually with time (Lampert & Sommer, 1997). Some of these species' substitutions can be observed in the current research compared with data of previous works, at Mequinenza, from species reported previously for summer season we found only two shared species. Larger filter species such as *N. alluaudi* and *D. galeata* together with the main predator *A. robustus*, were substituted for *C. numidicus* and *T. dybowskii*. A similar case occurred at Sobrón, where only three species were shared. From three cyclopids species to only *C. vicinus* and the presence of *D. galeata* and *C. pulchella*.

The reservoir with the most shared species was Oliana, with seven of the nine species reported for this study. The main change observed was *C. abyssorum* to *A. americanus*. The Sotonera reservoir was the only reservoir where the two previous copepod species did not suffer any variation, however, cladocerans from two *Daphnia* species changed to one species (*D. galeata*) and medium-size filters as *C. dubia* and *D. mongolianum*. The study of Higgins & Vander Zanden (2010) suggests that *D. polymorpha* can reduce the zooplankton biomass to 40-77 % in pelagic areas and replace them, this affect the species richness and diversity. The low replacement at Oliana could be related to the non-presence of them. In contrast, some changes can be appreciated at La Sotonera and Sobrón with low diversity (Fig. 3) for their increase in density and biomass (Table 3).

For the Ebro reservoir, there is a great difference in the number of zooplankton species between studies since previously 20 species were reported, where almost half of the species were microcrustaceans including several species of *Daphnia* and cyclopids, however, we registered only four shared species. Nowadays, only eight species are present, where five of them belong to rotifers and only *D. pulicaria* and *C. abyssorum* were reported before. Thus, all these data indicate that the communities have changed, increasing, or decreasing the number of species and being replaced for others. Several explanations such as competition, natural succession or even variations of environmental variables (Devetter, 1998; Dodson *et al.*, 2009) could explain these changes, however, since there is a lack of information for all non-reported years, the question of which exact events caused these changes remains unanswered.

Due to diverse factors, including management, most of water bodies cannot be sampled on a regular basis to confirm the species presents and like in this study, can take a long time until having new data. Nevertheless, having a monitoring program could help us to understand the community changes. But this is not the only benefit, thus, it can be a tool to have complete knowledge of species richness and to identify the already reported and the newly invasive species. For the invasive fauna, correct actions could prevent their introduction and dispersal along the watershed area, which could not only affect local diversity and become one of the major aquatic stressors, as is the case with zebra mussels (Strayer, 2010), but also create economic losses due to their impact on important infrastructures (Duran *et al.*, 2012).

The Zebra mussels were detected for first time at the Ebro watershed in 2001 (Duran & Anadón, 2008). Previously at La Sotonera reservoir the presence of *D. polymorpha* was not detected, however, now the veliger larvae can be found at both seasons and it's a dominant component of zooplankton. Thus, the mussel invasion has progressed throughout the years and among different reservoirs. The two reservoirs were mussels were not present are Oliana and Ebro, this last is under special protection (Duran & Anadón, 2008). Due to the lack of natural predators, efficient competition and non-intentional dispersion of invaders caused by the interaction between people among the reservoirs in the area, this invader could be detected in the Ebro reservoir in the upcoming years. Consequently, they would be present from the beginning until the end of watershed.

CONCLUSION

Our results show that abundances and biomass values were in general two times higher in summer than values in autumn. However, there is not an equal tendency for all reservoirs and each one works in a different way. The data suggest that the changes in the zooplankton community during both seasons are related mainly with physico-chemical variables as Chl-*a*, SD, TP, pH and reservoir depth, as well as with biotic interactions, like competition with alien species such as *D. polymorpha*. The relation between the zooplankton groups and the environmental variables could help us understand the main changes that could occur in a shifting world. All reservoirs presented new records in zooplankton species. La Sotonera had the highest number of new registers with ten species, followed by the Ebro with seven, while Oliana, Sobrón and Mequinenza have five. For Ullibarri-Gamboa reservoir we showed for the first time a record of zooplankton species. Also, we detected for first time the presence of zebra mussels at La Sotonera reservoir, indicating that this invader is dispersing throughout the watershed. Therefore, zooplankton composition knowledge, regular monitoring of species inhabiting in the reservoirs and the understanding of environmental variables that affect species and zooplankton structure (specific richness, density and biomass) can be a helpful tool for watershed management and early detection of invasive species.

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Chapter 2

Can zooplankton species be used as indicators of trophic status and ecological potential of reservoirs?

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Can zooplankton species be used as indicators of trophic status and ecological potential of reservoirs?

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Abstract The European Water Framework Directive implements the policies to achieve a good ecological status of all European waterbodies. To determine the ecological potential in freshwater environments, abiotic (morphology, physical and chemical variables) and biotics (algae, fishes, etc.) metrics are used. Despite their importance in trophic web, zooplankton was not included as one of the Biological Quality Elements (BQE) to determine the water quality. In the present research, we studied the zooplankton species that can be considered as indicators of trophic status and ecological potential for more than 60 water reservoirs. The data were obtained from more of 300 samples collected during 10 years from reservoirs at Ebro River watershed, which is the largest basin in Spain. According to their physico-chemical and biological elements, the trophic status and ecological potential of these reservoirs were established. More than 150 zooplankton species were identified during the study. The results from this research indicate that species that are related with low water quality are: *Acanthocyclops americanus*, *Ceriodaphnia* spp., *Daphnia cucullata*, *Daphnia párvula*, *Diaphanosoma brachyurum*, *Brachionus angularis*, *Keratella cochlearis* and *Phompolyx sulcata*. An indicator of moderate quality was *Bosmina longirostris*, while *Daphnia longispina*, *Ascomorpha ovalis* and *Ascomorpha saltans* were considered as indicators of good water quality. The data obtained suggest that zooplankton species can be used as a valuable tool to determine the water quality status and should be considered, in a near future, as one more of the BQE within the WFD metrics.

Keywords Bioindicators · European Water Framework Directive · Dams · Ebro watershed · Water quality

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Introduction

There is an ever-increasing pressure on water resources and freshwater cultural eutrophication (Schindler 2012). This cultural eutrophication is due to the increase in nutrient input (mainly nitrogen and phosphorus) directly into lakes, reservoirs, rivers or inside the catchment basin area. The nutrient increase is principally due to industrial activity and high human population growth and, increasing use of fertilizers in agriculture together with the effects of climate change, and can result in the degradation of inland waters (Moss 2011).

The European Water Framework Directive (Directive 2000) was introduced to present the requirements and assessments to control the water quality and classify the waterbodies into different "Ecological Status" in the European Union. The aim of the Water Framework Directive (WFD) is to achieve a "good ecological status" in all waterbodies. The classification of waterbodies is obtained through the unique hydro-morphologic, physical and chemical characteristics and a Biological Quality Element (BQE). The last parameter comprises benthic invertebrates, fish fauna, macrophytes, phytobenthos and phytoplankton. The BQE algae is one of the most used and accepted indicators to evaluate the ecological potential using plankton data. However, zooplankton, despite their fundamental position in food webs (Haberman and Haldna 2014) in freshwater ecosystems, was surprisingly not included (Moss 2007) and without a scientifically sound explanation for their omission (Caroni and Irvine 2010; Jeppesen et al. 2011; Moustaka-Gouni et al. 2014).

Zooplankton play an important role in energy transfer in trophic webs between primary producers and higher consumers and thus contribute significantly to nutrient recycling (Lampert and Sommer 1997). Due to their pivotal position in aquatic environments, the zooplankton community is strongly related with higher and lower levels of the trophic web. They can be affected by phytoplankton blooms during bottom-up processes and respond quickly (Jeppensen et al. 2011; Stamou et al. 2019) or apply pressure in the top-down control and determine the phytoplankton composition and abundance (Naselli-Flores and Rossetti 2010). Also, physical and chemical parameters such as temperature, dissolved oxygen, pH, conductivity and turbidity can determine species assemblages in the water column (Lampert 1997; Devetter 1998; Špoljar et al. 2018). Zooplankton thus have characteristics to be indicators of environmental conditions and trophic status (Anas et al. 2013; Kuczyńska-Kippen et al. 2020).

Several studies in the past pointed to zooplankton as useful indicators (Gulati 1983; Sládeček 1983; Berziņš and Pejler 1989). These days many authors have presented the utility of zooplankton as indicators of water quality and trophic state in water bodies using only one group of zooplankton such as rotifers (Duggan et al. 2001; May and O´Hare 2005; Ejsmont-Karabin 1995, 2012; Galir et al. 2018) or microcrustaceans (Boix et al. 2005; Pinto-Coelho et al. 2005; Haberman et al. 2007; Cheng et al. 2010; Ejsmont-Karabin and Karabin 2013; Jensen et al. 2013). Some other studies have considered both groups of zooplankton in general (Caroni and Irvine 2010; Jeppesen et al. 2011; Brito et al. 2011; Obertegger and Manca 2011; Haberman and Haldna 2014; Kehayias and Doulka 2014; Ochocka and Pasztaleniec 2016; Tasevska et al. 2017; Pociecha et al. 2018; Stamou et al. 2019).

In the Iberian Peninsula, recent studies have shown that zooplankton abundance (Garcia-Chicote et al. 2018) and community structure could be good indicators of trophic state in reservoirs in different basins, such as Jucar (Garcia-Chicote et al. 2019), Cavado (Almeida et al. 2020) and Ebro (Montagud et al. 2019). This last study presented the Zooplankton Reservoir Trophic Index (ZRTI) and can be considered as a preliminary approach to the present research. The reservoirs have a high importance in the socio-economic development of the Mediterranean region due to seasonal water scarcity. The main uses of these water resources are for human population requirements, large-scale agricultural irrigation and industrial use (Ibañez and Prat 2003; Cudennec et al. 2007).

The aim of this study was to determine the species of the three main zooplankton groups (rotifers, cladocerans and copepods) that are good indicators or are related to different trophic states in the reservoirs located in the Ebro watershed, using a robust data set collected during the last ten years in 66 reservoirs involving more than 300 sampling occasions. Also, following the guidelines of the WFD we assessed the water quality of the reservoirs and determined the species of zooplankton related to their ecological potential using the metrics specified in WFD, physicochemical and BQE algae metrics. The present research contributes to achieving a better zooplankton knowledge as water quality indicators by detecting key species related to trophic status and ecological potential.

Methods

Study site

The Ebro River is the largest river in Spain with a watershed area of 86,000 km^2, covering a fifth of the Spanish territory and one of larger basins in the Mediterranean region. The data presented in the current study were obtained from 66 reservoirs across the Ebro River watershed (Fig. 1) during summers of 2010 to 2019. To collect the corresponding samples in each reservoir, a sampling point was established in the deepest part of the reservoir at 300–500 m from the dam.

Environmental parameters, trophic state and ecological potential

At every sampling point, the following variables were measured in situ along a vertical profile: dissolved oxygen, temperature, conductivity, turbidity, pH and chlorophyll-*a* using a multisensory devise Sea-Bird 19 plus V2 (Seabird®, USA). The photic zone depth was calculated measuring the light penetration using a Li-Cor quanta-meter. The water transparency was determined measuring the Secchi disk depth (SD). For ex situ analysis, an integrative water sample was collected from the photic zone of each reservoir using a 25-mm-inner-diameter ballasted PET tube, and when photic zone was lower than 6 m deep, an integrative water sample was collected from the water surface until this depth or to the bottom (Vicente et al. 2005). We used standard methodology for estimating the following variables: suspended solids, turbidity, total phosphorus (TP) and chlorophyll *a* (Shoaf and Lium 1976; APHA 1998).

To determine the trophic state of each reservoir, we used the Trophic State Index (TSI') (Carlson 1977). To obtain a final trophic state, we used the average of the three variables of TSI´ (total phosphorus, chlorophyll-*a* and Secchi disk depth).

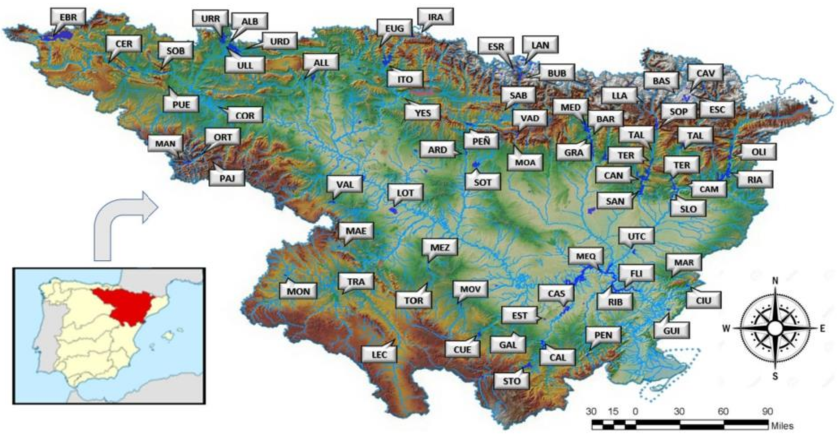

Fig. 1 Map of Ebro watershed with the approximate location of studied reservoirs. ALB Albiña, ALL Alloz, ARD Ardisa, BAL Balaguer, BAR Barasona, BAS Baserca, BUB Búbal, CAL Calanda, CAM Camarasa, CAN Canelles, CAS Caspe, CAV Cavallers, CER Cereceda, CIU Çiurana, COR El Cortijo, CUE Foradada, EBR Ebro, ESC Escales, ESR Escarra, EST Alcañiz, EUG Eugui, FLI Flix, GAL Gallipuén, GRA El Grado, GUI Guiamets, IRA Irabia, ITO Itoiz LAN Lanuza, LEC Lechago, LLA Llauset, LOT La Loteta, MAE Maidevera, MAN Mansilla, MAR Margalef, MED Mediano, MEQ Mequinenza, MEZ Mezalocha, MOA Montearagon, MON Vicarías, MOV Moneva, OLI Oliana, ORT Ortigosa, PAJ Pajares, PEÑ La Peña, PEN Pena, PUE Puentelarra, RIA Rialb, RIB Ribarroja, SAB Sabiñanigo, SAN Santa Ana, SLO San Lorenzo, SOB Sobrón, SOP Soperia, SOT Sotonera, STO Santolea, TAL Talrn, TER Terradets, TOR Las Torcas, TRA Tranquera, ULL Ullivari, URD Urdalur, URR Urrunaga, UTC Utexa seca, VAD Vadiello, VAL Val, YES Yesa

The ecological potential (EP) was calculated following the methodology in "Spanish Legislation RD 817/2015" and Directive (2000). To obtain the EP, both biological and physicochemical indicators were assessed. The biological indices were obtained using the metrics obtained from four algal variables (chlorophyll *a*, biovolume, percentage of cyanobacteria and the IGA Index (Catalan and Ventura 2003)). Based on these, the classification scheme was Good or Superior, Moderate, Poor and Bad. The physicochemical indicator was obtained from the Secchi disk depth, hypolimnetic oxygen concentration and total phosphorus as variables. Their respective classifications were Very Good, Good, Moderate, Poor and Bad. To establish the representative classification of each biological and physicochemical indicator, we selected the average value of the algae and physicochemical variables. Following the WFD procedure using the "one-out, all-out" rule, the worst value between both indicators was selected as the ecological potential. A detailed methodology to obtain the ecological potential can be found in CHE 2016. In addition to determining the ecological potential using the standard procedure, we used the two previous indicators individually as the ecological potential to verify if there is a difference in the composition of zooplankton species classified as indicators.

Zooplankton samples

The zooplankton samples were collected using a vertical Ruttner bottle with capacity of 2.7 L. In each waterbody, we took two Ruttner bottles to obtain 5.4 L of sample; afterwards, it was filtered through a 30-μm mesh size Nytal. Also, a zooplankton vertical tow net of 45-μm mesh size Nytal was towed from 30 m depth or from the reservoir bottom until the surface; these vertical tow net samples were collected mainly for taxonomic purposes. Both vertical and Ruttner samples were fixed with formalin at 4% final concentration and stored in a hermetic glass vial. The depth at which the zooplankton samples were collected was established for each reservoir at the beginning of oxycline, which has been reported as the richest zone of zooplankton fauna (Miracle and Vicente 1983).

The zooplankton species were identified using Ruttner-Kolisko (1974), Koste (1978), Nogrady et al. (1995) and Nogrady and Segers (2002) for rotifers, Alonso (1996) and Błedzki and Rybak (2016) for microcrustaceans. Since we detected the presence in several reservoirs of the veliger larvae of invader bivalve zebra mussel (*Dresseina polymorpha*), we counted them for further studies. The samples obtained from Ruttner samples were counted using a Sedgewick Rafter-type chamber (1 mL) under inverted microscope (Nikon Eclipse Ti-U, objective lens 4x-60 $\times$ DIC) to obtain the corresponding specific richness, species abundance and biomass.

Data analysis

A total of 304 samples were collected during 10 years of sampling. We considered each sample obtained as a datum, corresponding to the reservoir and date that was sampled. Using the total of zooplankton species presented in all reservoirs, we ran a similarity percentage analysis (SIMPER) to identify the species that most contributed to changes inside the communities. The SIMPER analysis was performed with the Bray–Curtis index with zooplankton abundances using PAST software (Hammer et al. 2001). To determine the relationship between environmental variables and zooplankton species, we ran a Canonical Correspondence Analysis (CCA). This analysis was performed using abundance data of zooplankton dominant species that are those species that were > 0.1% of the total zooplankton individuals (Table 1); also the species that were only present in only one reservoir were not included in the analysis. For this analysis, the selected environmental variables were: chlorophyll *a*, total phosphorus, turbidity, suspended solids, temperature, conductivity, dissolved oxygen, pH, Secchi disk depth and water residence time All the data, except pH, were normalized transformed logarithmically Log $(x + 1)$. The model was tested using a Monte Carlo permutation (n = 999). The CCA was performed using the CANOCO 4.5 program for Windows system (ter Braak and Šmilauer 2002).

A second evaluation of indicator species related with trophic state and ecological potential was carried out using the Indicator Value (IndVal). This method uses and combines the species relative abundance (specificity) with the relative frequency of occurrence (fidelity) of the species in different habitats. The IndVal arranges the species in groups and gives values between 0 and 1; those species with values $\geq$ 0.50 and significance ($p < 0.05$) can be used as indicators (Dufrene and Legendre 1997; Cáceres and Legendre

Table 1 Dominant zooplankton species > 0.1% of total density

Code	Zooplankton Species	Code	Zooplankton Species
P1	*Acanthocyclops americanus*	R9	*Conochilus natans*
P2	*Copidodiaptomus numidicus*	R10	*Conochilus* sp.
P3	*Cyclops* sp.	R11	*Conochilus unicornis*
P4	*Cyclops vicinus*	R12	*Gastropus stylifer*
P5	*Eudiaptomus vulgaris*	R13	*Hexarthra fennica*
P6	*Neolovenula alluaudi*	R14	*Hexarthra intermedia*
P7	*Thermocyclops dybowskii*	R15	*Hexarthra mira*
P8	*Tropocyclops prasinus*	R16	*Hexarthra oxyuris*
C1	*Bosmina longirostris*	R17	*Kellicotia longispina*
C2	*Ceriodaphnia dubia*	R18	*Keratella cochlearis*
C3	*Ceriodaphnia pulchella*	R19	*Keratella cochlearis* f. *tecta*
C4	*Daphnia cucullata*	R20	*Keratella quadrata*
C5	*Daphnia galeata*	R21	*Polyarthra dolichoptera*
C6	*Daphnia longispina*	R22	*Polyarthra euryptera*
C7	*Daphnia parvula*	R23	*Polyarthra luminosa*
C8	*Daphnia pulicaria*	R24	*Polyarthra major*
C9	*Diaphanosoma brachyurum*	R25	*Polyarthra vulgaris*
C10	*Diaphanosoma mongolianum*	R26	*Pompholyx sulcata*
C11	*Diaphanosoma* sp.	R27	*Synchaeta kitina*
R1	*Anuraeopsis fissa*	R28	*Synchaeta longipes*
R2	*Ascomorpha ovalis*	R29	*Synchaeta oblonga*
R3	*Ascomorpha saltans*	R30	*Synchaeta pectinata*
R4	*Asplanchna girodi*	R31	*Synchaeta stylata*
R5	*Asplanchna priodonta*	R32	*Trichocerca pusilla*
R6	*Collotheca pelagica*	R33	*Trichocerca similis*
R7	*Collotheca* sp.	Dp	*Dreissena polymorpha*
R8	*Conochilus dossuarius*		

P = Copepoda species, C = Cladocera species, R = Rotifera species

2009). The analysis was performed with the *indicscpecies* package using R 4.0.0 "Arbor day" version (R Core Team 2020).

Results

Studied reservoirs, trophic state and ecological potential

Sampled reservoirs were classified by their trophic state according to the TSI´ (Carlson 1977); then, samples were classified as: 123 oligotrophic, 123 mesotrophic, 55 eutrophic and only 3 as hypereutrophic. Following WFD protocols, sampled reservoirs were assessed using both physicochemical and biological metrics to obtain their final ecological potential; samples were classified as: 99 Good or Superior, 202 Moderate and only 3 as Poor. Considering only the physicochemical metrics as final ecological potential, the results were the same as above. On the other hand, using only the algae metrics, the ecological potential of sampled reservoirs was better: 273 were Good or Superior, 28 Moderate and only 3 as Poor. The complete information related with the reservoirs trophic state and ecological potential can be found in Supplementary Table 1.

Zooplankton assemblage

During this study, 169 zooplankton species were identified. The rotifers species richness was the highest (115) followed by cladocerans (36) copepods (17) and the veliger larvae of zebra mussel (*D. polymorpha).* The complete zooplankton species list can be found in Supplementary Table 2. The reservoir with the highest zooplankton richness was Santolea in the year 2010 with 26 species, where 18 species belong to rotifers; also was the reservoir with rotifera higher richness. The cladocera major richness was present in six reservoirs; in each of these reservoirs were found six cladocera species. In the case of copepods, two reservoirs presented higher species richness with five species each.

The richness found in eutrophic reservoirs was similar to oligotrophic reservoirs: oligotrophic 10.6 ± 3.3, mesotrophic 12.2 ± 4, eutrophic 11.2 ± 3.7 and hypereutrophic 11 ± 1.7. The same tendency can be seen when classifying the reservoirs using the WFD metrics: Good or Superior 10.8 ± 3.9, Moderate 11.6 ± 3.5, Poor 16.5 ± 0.5 and Bad 10.

Statistical interpretation

The results from SIMPER analysis were divided in two steps. First, we ran the analysis using all trophic state data (oligotrophic until hypereutrophic) from all reservoirs, and next, we used the data of only maximum and minimum trophic state reservoirs. The same procedure was performed for ecological potential, using first the complete data, and then only the higher and lower potential. The species *P. dolichoptera*. *K. cochlearis*, *P. major*, *A. americanus*, *D. cucullata* and *D. polymorpha* were responsible of major variance in trophic state and ecological potential among reservoirs (Table 2).

The relationship between the selected environmental variables and the dominant zooplankton species carried out with the CCA showed that the first two ordination axes explain the 58.2% of variance, with a *p* value of 0.001 in the Monte Carlo permutation test. The first axis (39.8%) is related with the trophic state variables. Its positive part is related to oligotrophic variables as Secchi disk depth, and the species related were the cladoceran *D. longispina* and rotifers *Trichocerca similis*, *Gastropus stylifer*, *Ascomorpha saltans* and *A. ovalis*. The eutrophic elements such as chlorophyll and total phosphorus are negatively correlated with this axis. The species that show a strong correlation with these eutrophic variables were the copepods *A. americanus* and *Cyclops vicinus,* cladocerans *B. longirostris, D. cucullata, Daphnia parvula* and rotifers *Pompholyx sulcata*, *Hexarthra intermedia*, *K. cochlearis* f. *tecta*, *Polyarthra vulgaris*, *Polyarthra euryptera* and *Asplanchna girodi* (Fig. 2).

The second axis (18.4%) explains the relationship among environmental variables and zooplankton species; in the negative part several species are related with conductivity, principally *Tropocyclops prasinus*, *Copidodiaptomus numidicus*, *Hexarthra fennica*, *Ceriodaphnia dubia* and *Diaphanosoma mongolianum.* The dissolved oxygen was related to some species such as *Diaphanosoma brachyurum*, *Synchaeta stylata*, *Synchaeta longipes*, *Conochilus unicornis* and *Anuraeopsis fissa*.

The Indicator Value analysis (IndVal; Table 3) using the data from the reservoirs trophic state indicates the presence of 14 species with significant values ($p < 0.05$); most of these species (11) were indicators of hypereutrophic status, while the rest of species were related to transition states: one as Eu–hypereutrophic, one as meso–eutrophic and one as oligo–mesotrophic.

The results of the IndVal with the ecological potential data exhibit that indicator species decreased to six: one species as Good or Superior and five as Poor. Also, we ran another IndVal using only the data from the algae metrics. The results obtained with this new analysis provide nine indicator species, two as Poor, two as Poor–Moderate and five as Moderate (Table 4).

Discussion

Through the statistical treatment applied in the present research to the large dataset obtained along the largest basin in Spain, we have been able to define the zooplankton species that are capable of being good indicators of different environmental conditions and trophic status. Some of these species can be used also to determine the water quality and ecological potential within the WFD.

The trophic status in reservoirs normally exhibits similar tendencies over the years; for example, Mequinenza was oligotrophic or mesotrophic for most

Table 2 SIMPER analysis results

Simper											
Trophic State			Oligotrophic versus hypereutrophic			Ecological potential			Good or superior versus bad		
Species	Contrib. %	Cumulative %	Species	Contrib. %	Cumulative %	Species	Contrib. %	Cumulative %	Species	Contrib. %	Cumulative %
P. dolichoptera	18.85	18.85	*A. americanus*	14.46	14.16	*P. dolichoptera*	18.85	18.85	*K. cochlearis*	34	34
K. cochlearis	9.65	28.5	*D. cucullata*	13.6	27.76	*K. cochlearis*	10.22	29.07	*P. dolichoptera*	21	55
D. polymorpha	8.13	36.63	*P. major*	12.2	39.96	*P. major*	7.68	36.75	*A. ovalis*	7.3	62.3
P. major	7.97	44.6	*P. dolichoptera*	8	47.96	*D. polymorpha*	6.7	43.45	*P. luminosa*	4.6	66.9

Contrib % = variance contribution percentage of each species

of time. The only three reservoirs classified as hypereutrophic were Mezalocha 2012, Utxesa 2016 and Moneva 2017, due to an increase in the values of chlorophyll *a*, total phosphorus and low Secchi Disk transparency compared with previous or later years. In the case of the first two reservoirs, the rest of the years were classified as eutrophic; however, Moneva ranged between oligotrophic and hypereutrophic throughout the monitoring period. Despite the increase in their trophic state, the ecological potential of these three reservoirs was cataloged as Moderate, regardless of the use of physicochemical or algae metrics. The low sensitivity of these variables leads to the opportunity to test other biological strategies with higher sensibility, such as zooplankton, to obtain more precise results.

Worldwide, algae are one of the most accepted groups to obtain metrics to assess trophic conditions and water quality due to the dynamics of their species assemblage, functional groups, density and response to environmental conditions (Reynolds et al. 2002; Padisák et al. 2006). Also, several algae metrics were established to be reassessed within the WFD, such as biovolume, composition and chlorophyll *a* (Ptacnik et al. 2008; Poikane et al. 2009; Phillips et al. 2013). The zooplankton can also provide valuable information with various types of metrics to determine the trophic conditions, i.e., functional groups (Obertegger and Manca 2011; Sun et al. 2019; Kuczynska-Kippen et al. 2020), density (May and O´Hare 2005; Ejsmont-Karabin 2012; Ejsmont-Karabin and Karabin 2013) and species composition (Attayde and Bozelli 1998; Pinto-Coelho et al. 2005; Montagud et al. 2019; Muñoz-Colmenares et al. 2021).

Species composition and their relationship with the environmental variables through the CCA analysis indicate that the set of variables related to eutrophic conditions as chlorophyll *a* and total phosphorus together with suspended solids and turbidity were decisive for the presence of a significant number of species such as *A. americanus*, *D. parvula*, *P. sulcata*, *K. cochlearis* f. *tecta* and *A. girodi;* these species were reported also as eutrophic species in the ZRTI index (Montagud et al. 2019). While species such as *B. longirostris* and *D. cucullata* have been related to meso-eutrophic environments (Haberman et al. 2007; Jensen et al. 2013), the species from our analysis related to Secchi disk and oligotrophic state including *D. longispina*, *T. similis*, *G. stylifer*, *A. saltans* and *A*

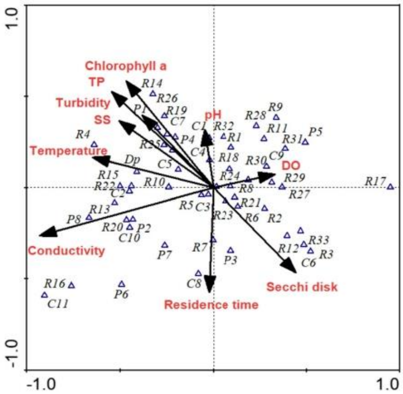

Fig. 2 Canonical Correspondence Analysis of dominant zooplankton species and environmental variables. TP = total phosphorus, SS = suspended solids, DO = dissolved oxygen. Zooplankton species codes names are as denoted in Table 1

ovalis were also reported in the ZRTI as oligo-mesotrophic species. Besides, temperature exhibits a strong relation with the species *A. girodi*, *P. euryptera*, *H. mira*, *C. dubia* and *D. polymorpha*; this variable was located near variables of high trophic states. Recently, it has been suggested that temperature can be responsible of community composition and size structure of cladocerans (Haberman et al. 2007), rotifers (Chalkia and Kehayias 2013) and zooplankton metrics as abundance and biomass, especially in a global warming scenario (Cremona et al. 2020; Dziuba et al. 2020). The use of CCA analysis together with other biological indicators methods such as IndVal and SIMPER analysis can be useful to determine properly the species associated with certain environments, habitats and highlight the differences between the fauna present in different trophic state and ecological potential levels.

The use of indicator value of species, to assess water quality, community preferences and pollution levels, has been widely used in diverse aquatic environments. Some examples of applying this IndVal with diverse aquatic groups are its use with fishes in the Mediterranean Sea (Carlucci et al. 2018), macrophytes in urban reservoirs (Silva et al. 2014), plankton groups in alpine lakes (Catalan et al. 2009), diatoms in saline lakes (Stenger-Kovács et al. 2014), marine zooplankton (Mazzocchi et al. 2011) and recently freshwater zooplankton to determine the trophic state in reservoirs in Spain (Garcia-Chicote et al. 2019).

The rotifers *A. girodi*, *P. sulcata* and *K. tropica* reported by Garcia-Chicote et al. (2019) at Jucar watershed as indicators of high trophic status are in accordance with our IndVal results (Table 3); however, their results do not show any species related to oligotrophic conditions; meanwhile, our data suggest that *D. longispina* indicate the oligo-mesotrophic status. The characterization of this low trophic indicator species probably is due to the difference in the trophic state of reservoirs, since in the present study the proportion of oligotrophic reservoirs was higher than in the Jucar study. This same cladoceran *D. longispina* was the only indicator of a Good or Superior status; meanwhile, some species related with low ecological levels were similar to those related with high trophic states such as *A. americanus* and *K. cochlearis*. Using only the algae metrics, there is no species with good potential; in contrast, some species catalogued previously as indicators of high trophic condition and low ecological potentials are located as indicators of Moderate state such as *C. dubia,* which has been reported as tolerant to eutrophication (Azevêdo et al. 2015). The total number of indicator species was higher using the trophic state than the elements of the WFD, so this suggests that zooplankton can be more sensitive to changes in trophic status than in ecological potential.

In oligotrophic waterbodies, the zooplankton density and biomass are lower compared with those reported with higher trophic status (Lampert and Sommer 1997; May and O´Hare 2005; Garcia-Chicote et al. 2018). The IndVal method is based principally on the detection of species density associated with certain variables or particular conditions (i.e., trophic state); therefore, applying this method to zooplankton can be reliable to determine the species related especially with higher trophic levels or associated with low ecological potential status.

Normally, as trophic state increases, zooplankton large filter species decrease considerably and are replaced by smaller-sized species (Jeppesen et al. 2000; Pinto-Coelho et al. 2005). A similar tendency was detected in the SIMPER results comparing the lowest and highest trophic level. The small sized species were responsible for community change among oligotrophic to eutrophic–hypereutrophic

Table 3 Indicator value (IndVal) from zooplankton species and trophic state, only those species with $p < 0.05$ are shown

Species	IndVal	p value	Trophic state
Acanthocyclops americanus	0.938	0.0002	Hypereutrophic
Polyarthra major	0.837	0.0077	Hypereutrophic
Pompholyx sulcata	0.808	0.0027	Hypereutrophic
Daphnia cucullata	0.805	0.0058	Hypereutrophic
Cyclops abyssorum	0.781	0.0024	Hypereutrophic
Keratella cochlearis f. *tecta*	0.702	0.0119	Hypereutrophic
Lecane stichaea	0.574	0.0062	Hypereutrophic
Cyclops vicinus	0.544	0.0325	Hypereutrophic
Keratella tropica	0.525	0.0353	Hypereutrophic
Asplanchna girodi	0.518	0.0257	Hypereutrophic
Daphnia parvula	0.499	0.0480	Hypereutrophic
Bosmina longirostris	0.829	0.0102	Eu-Hypeurtrophic
Keratella quadrata	0.510	0.0485	Meso-Eutrophic
Daphnia longispina	0.616	0.0500	Oligo-Mesotrophic

Table 4 Indicator value (IndVal) of zooplankton species and ecological potential using WFD (water framework directive) and algae metrics. Only those species with $p < 0.05$ are shown

Species	IndVal	p value	Ecological potential
WFD			
Keratella cochlearis	0.836	0.032	Poor
Collotheca pelagica	0.654	0.009	Poor
Acanthocyclops americanus	0.571	0.011	Poor
Brachionus angularis	0.562	0.011	Poor
Diaphanosoma brachyurum	0.552	0.016	Poor
Daphnia longispina	0.683	0.045	Good or superior
Algae metrics			
Collotheca pelagica	0.703	0.009	Poor
Diaphanosoma brachyurum	0.541	0.039	Poor
Bosmina longirostris	0.848	0.017	Poor–moderate
Keratella cochlearis	0.828	0.039	Poor–moderate
Acanthocyclops americanus	0.788	0.015	Moderate
Keratella cochlearis f. *tecta*	0.734	0.015	Moderate
Pompholyx sulcata	0.668	0.013	Moderate
Daphnia cucullata	0.641	0.033	Moderate
Ceriodaphnia dubia	0.585	0.034	Moderate

reservoirs, and some of small species are also shared in the high eutrophic results of CCA and in the IndVal test. Some of these species were the rotifers *P. sulcata, K. cochlearis, A. girodi*, cladoceran *D. cucullata* and the cyclopoid copepod *A. americanus* that are consider typical from eutrophic waters (Attayde and Bozelli 1998; Duggan et al. 2001; Smakulska and Górniak 2004; Haberman et al. 2007; Kehayias and Doulka 2014). The use of multiples tests, as CCA, SIMPER and Indval, together with species that are present and shared among them, can give us a more precise acquaintance data about species that have high potential to be used as indicators.

Cyclopoid copepods are more abundant in high trophic environments in temperate and tropical regions (Pinto-Coelho et al. 2005). In concordance with our results, *A. americanus* has been reported in eutrophic waterbodies in Spain (Garcia-Chicote et al. 2019; Montagud et al. 2019) and Mexico (Nandini et al. 2016). Besides, other *Acanthocyclops* spp. and *Cyclops* genera can be found worldwide in meso- to eutrophic waters, such as Asia (Chengxue et al. 2019), Oceania (Duggan et al. 2020), Europe (Haberman et al. 2014) and South America (Perbiche-Neves et al. 2016).

In contrast, large filtering microcrustacean such as calanoid copepods and large *Daphnia* species are found worldwide in low production waters, as in Europe (Ejsmont-Karabin and Karabin 2013; Stamou et al. 2019; Montagud et al. 2019), North America (Pinto-Coelho et al. 2005; Muñoz-Colmenares et al. 2017) and South America (Brito et al. 2011; Picapedra et al. 2020) together with the rotifers *A. ovalis* and *A. saltans* (Montagud et al. 2019; Duggan et al. 2020) that are present in the current research.

Previously, to determine the ecological potential using phytoplankton the follow metrics are used under the WFD criteria: biovolume, chlorophyll *a*, Catalán index (IGA) and percentage of cyanobacteria. In the case of zooplankton, it should have their own methodology comparable to those in phytoplankton. We suggest that similar metrics for zooplankton could be the species that in our present research were found as indicators of different trophic state and ecological potential levels along with the species provided in the ZRTI index (Montagud et al. 2019). Besides, the use of abundance and biomass of zooplankton groups (Garcia-Chicote et al. 2019) could be a good complementation. For sample collection, a standard methodology would work good, quantitative samples using Ruttner bottles or any other technique that permits obtain accurate numerical estimations that lead the correct implementation of metrics and indexes.

The integrative capacity of zooplankton species of the environmental factors that determine the trophic state and the ecological potential gives us a broader picture over time compared to phytoplankton. Since this last group has a shorter life span and their communities can vary in less time compared to zooplankton (Reynolds, 2006) and sometimes under specific environmental pressures, the phytoplankton could not give a so accurate picture of how the aquatic system is really in general. However, the use of both phytoplankton and zooplankton species present in the waterbodies can be complementary and would give us a more precise picture of the water quality, trophic state or ecological potential. Zooplankton sample collection is not complicated and generally can be taken at the same time with phytoplankton and can be included in monitoring programs easily. Thus, we recommend with great emphasis, as many other authors before us, that zooplankton should be included as one more of BQE for Water Framework Directive.

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Availability of data and material Data are available at public site http://www.chebro.es.

Code availability All used packages and software tools have been cited properly in the manuscript. There is not a specific code.

Declarations

Conflict of interest The authors have no conflicts of interest or competing interest.

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Chapter 3

The Use of Zooplankton Metrics to Determine the Trophic Status and Ecological Potential: An Approach in a Large Mediterranean Watershed.

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The Use of Zooplankton Metrics to Determine the Trophic Status and Ecological Potential: An Approach in a Large Mediterranean Watershed

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Abstract: In the European Water Framework Directive, zooplankton was not included as a Biological Quality Element despite its important place in the aquatic trophic web. In the present study on zooplankton abundances and biomasses, we used several metrics to test their ability to detect differences among trophic statuses and ecological potential levels, and collected a large sum of data in more than 60 reservoirs at Ebro watershed, on more than 300 sampling occasions over 10 years. Our results indicate that most zooplankton metrics are correlated to environmental variables that determine reservoirs' trophic states, especially chlorophyll *a* and total phosphorus. The metrics with better sensitivity to differentiate trophic states and ecological potential levels were ZOO (total zooplankton), LZOO (large zooplankton), CLAD (cladocerans), and ZOO:CHLA (zooplankton:chlorophyll *a* ratio). Microcrustacean metrics such as DAPHN (*Daphnia*), COP (copepods), CYCLO (cyclopoids), and CALA (calanoids) were good at differentiating between high and low water quality in trophic status (oligotrophic–eutrophic) and ecological potential (good or superior–moderate). Thus, zooplankton can be used as a valuable tool to determine water quality; we believe that zooplankton should be considered a Biological Quality Element within Water Framework Directive monitoring programs for inland waters.

Keywords: bioindicators; biological quality element; reservoirs; water framework directive; ebro watershed

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1. Introduction

Water resources such as natural lakes and man-made reservoirs have been vital in supporting the increase in population growth, agricultural productivity, industrial activity, and economic development [1]. Presently, a high pressure on water resources is present around the world due to the previous factors in unison with climate change and freshwater cultural eutrophication [2]. This cultural eutrophication is mainly an input increment of nitrogen and phosphorus into waterbodies or catchment basin areas, and primarily caused by industrial activity [3]. These high inputs and rising temperatures tend to intensify eutrophication and lead to cyanobacterial blooms, floating plant predominance, dissolved oxygen decrement, and, therefore, low water quality [4].

The implementation of the European Water Framework Directive was one response to this situation. This directive presents the requirements and assessments to control the water quality and classify waterbodies into different "Ecological Status" throughout the European Union [5]. The main aim of the Water Framework Directive (WFD) is to achieve a "good ecological status" in all waterbodies. This ecological status is obtained through hydro-morphological and physical–chemical indicators, as well as certain Biological Quality Elements (BQE). Included in the last indicators were phytoplankton, phytobenthos, macrophytes, benthic macroinvertebrates, and fish. However, and, surprisingly, without a scientifically based explanation, zooplankton was not included as a BQE [6–8].

Zooplankton organisms have a fundamental position in aquatic food webs [9] and are an important element in the structure and function of aquatic ecosystems. This is because they occupy the role of primary consumers and are the energy link between primary producers such as phytoplankton and higher consumers such as fish [10]. Additionally, zooplankton can respond quickly to changes from trophic cascades, such as phytoplankton blooms, in a bottom-up process or top-down control, controlling and determining algae composition and abundance [11–13]. Moreover, the zooplankton community responds to physical–chemical habitat conditions easily, which affects their species' richness, increasing or decreasing densities, and promoting shifts in their diversity [14,15]. It is because of these characteristics that they can be a suitable indicator of water quality [16,17].

Worldwide, in waterbodies with different environmental conditions, several studies have used the whole zooplankton community as an indicator [7,18–21] or used only specific zooplankton groups, such as rotifers or microcrustaceans [22–25]. Recently, within the Iberian Peninsula, the use of zooplankton species as indicators of trophic status in reservoirs has been evaluated in different basins such as Ebro [26,27], Cavado [28], and Jucar [29].

Studies focused on the use of zooplankton biomass, abundance, and ratios to determine trophic state have recently increased in several parts of the European Union [8,9,30–33]. However, in natural lakes [34–36] and man-made reservoirs in the Mediterranean region, there are fewer studies [37,38]. Finally, studies on the use of zooplankton biomass and abundance as indicators of ecological status under the WDF criteria are scarce in scientific research.

The aim of this study was to establish the value of the zooplankton metrics used in determining the trophic status and ecological potential for lentic waters. In the study, we used a robust data set collected over the last ten years in 66 reservoirs along the Ebro watershed, involving more than 300 sampling events over 10 years. The present research contributes to the research on zooplankton as a useful indicator to determine the trophic state and ecological potential within the context of the WFD requirements.

2. Materials and Methods

2.1. Study Site

The largest river in Spain is the Ebro River; it has a draining area of 86,000 km^2, covering a fifth of the Spanish territory, and it is one of the larger Mediterranean watersheds. The Ebro River flows 930 km from the northwest to the southeast and before joining with the Mediterranean Sea at Amposta, which is located approximately 160 km south of Barcelona. The data presented in this work was obtained from 66 different reservoirs across the Ebro watershed (Figure 1). According to WFD methodology, sampling campaigns were conducted during the summers from 2010 to 2019. At each reservoir, a sampling point was established to collect environmental data, water, and plankton samples. This point was set up in each reservoir's deepest part, at 300–500 m from the dam wall. Some data and characteristics of each reservoir are presented in Appendix A, Table A1, and Supplementary Table S1.

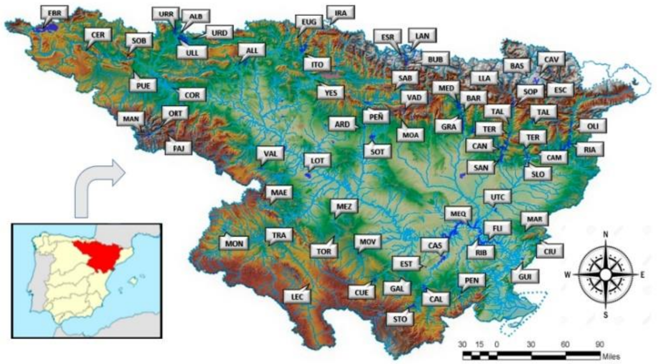

Figure 1. Map of Ebro watershed with approximate location of the studied reservoirs. Reservoir codes are listed in Appendix A.

2.2. Environmental Variables

At every sampling point, the following variables were measured in situ along a vertical profile: dissolved oxygen, conductivity, temperature, turbidity, chlorophyll-*a*, and phycocyanin, among many others, using a multisensory device called Sea-Bird 19 plus V2 (Sea Bird Electronics Inc., Bellevue, WA, USA). The water transparency was determined through the Secchi disk depth (SD). For ex-situ analysis, an integrative water sample was collected from the photic zone of each reservoir using a 25 mm inner diameter ballasted PET tube. The depth of the photic zone was calculated by means of the light penetration using a Li-Cor quantum meter (LI-COR Environmental, Lincoln, NE, USA). When the photic zone was less than 6 m deep, the integrative water sample was collected from the water's surface down to said depth or to the bottom [39]. We used standard methodology to estimate the total phosphorus (TP), total nitrogen (TN), and chlorophyll *a* (Chl-*a*) [40,41].

2.3. Trophic State and Ecological Potential

To determine the trophic state in each reservoir we used the Trophic State Index (TSI′) [42]. To obtain a final trophic state we used the average of the three variables of TSI′ (TP, Chl-*a*, and Secchi disk). The ecological potential (EP) was calculated according to methodology in "Spanish Legislation RD 817/2015" and WFD [5], using the biological and physicochemical indicators. The biological indices were obtained using the metrics taken from four algal variables (chlorophyll *a*, biovolume, percentage of cyanobacteria, and the Index of Algae Groups [43]). From these, the classification scheme was: Good or Superior, Moderate, Poor, and Bad. The physicochemical indicator was obtained from the Secchi disk depth, hypolimnetic oxygen concentration, and total phosphorus as variables. From these, the respective classifications were Very Good, Good, Moderate, Poor, and Bad. To establish the representative classification of each biological and physicochemical indicator, we selected the average value of the algae and physicochemical variables. Following the WFD procedure, using the "one-out, all-out" rule, the worst value between both indicators was selected as ecological potential. A detailed methodology to obtain the ecological potential can be found in C.H.E. [44].

2.4. Zooplankton Samples

Zooplankton samples were collected at the beginning of the oxycline, which is reported as the richest zone of zooplankton fauna during the day [45]. Samples were collected using a vertical Ruttner bottle with 2.7 L capacity. For each reservoir, we took two Ruttner bottles to obtain 5.4 L of water sample, which was then filtered through a 30 µm mesh size Nytal. In addition, for taxonomic purposes, a zooplankton vertical tow net of 45 µm mesh size Nytal was towed from either a depth of 30 m, or the bottom of the reservoir, to the surface. Once both samples were obtained, they were fixed with formalin at 4% final concentration and stored in hermetic glass vials.

Zooplankton species were identified using Koste [46], Nogrady and Segers [47], and Ruttner-Kolisko [48] for rotifers; Alonso [49] and Błedzki and Rybak [50] for microcrustacean groups. The samples obtained from the Ruttner bottles, were counted using a 20 mL sedimentation chamber under inverted microscope (Nikon Eclipse Ti-U, objective lens 4 × −60, with differential interference contrast (DIC)). Zooplankton biomass was estimated for each taxon using a minimum of 30 specimens that were measured to calculate their dry weight using biovolume and length–weight relationships [51–53].

2.5. Zooplankton Metrics

The selected metrics, used to test the zooplankton viability to determine trophic state and ecological potential, were performed separately with the abundances and biomass of:

(a) ZOO (zooplankton in total (rotifers, copepods, and cladocerans together) [8,38])
(b) LZOO (large zooplankton (advanced copepods stages and large cladocerans) (Table 1) [54])
(c) SZOO (small zooplankton (rotifers, nauplii, and small cladocerans) [54])
(d) ZOO:CHLA (Zooplankton:chlorophyll *a* ratio [54])
(e) ZOO:PHYTO (Zooplankton:phytoplankton ratio [55])
(f) Zooplankton major groups: ROT (rotifers), CLAD (cladocerans), and COP (copepods) [31,32]
(g) Selected microcrustacean orders/genera: DAPHN (daphnids), CYCLO (cyclopoids), and CALA (calanoids) [32]

Table 1. Zooplankton large and small bodied genera found in the present study.

Large		**Small**			
Cladocera	**Copepoda**	**Cladocera**	**Rotifera**		
Daphnia	*Acanthocyclops*	*Alona*	*Anuraeopsis*	*Gastropus*	*Ploesoma*
Diaphanosoma	*Cyclops*	*Alonella*	*Ascomorpha*	*Hexarthra*	*Polyarthra*
Holopedium	*Eucyclops*	*Bosmina*	*Asplanchna*	*Hexarthra*	*Phompolyx*
Ilyocryptus	*Macrocyclops*	*Ceriodaphnia*	*Brachionus*	*Kellicottia*	*Proales*
Leydigia	*Thermocyclops*	*Chydorus*	*Cephalodella*	*Keratella*	*Ptygura*
Macrothrix	*Tropocyclops*	*Moina*	*Collotheca*	*Lecane*	*Squatinella*
Sida	*Copidodiaptomus*	*Oxyurella*	*Colurella*	*Lepadella*	*Synchaeta*
	Eudiaptomus		*Conochilus*	*Lophocaris*	*Testudinella*
	Neolovenula		*Dicranophorus*	*Macrochaetus*	*Trichocerca*
	Ergasilus		*Encentrum*	*Monommata*	*Trichotria*
	Neoergasilus		*Eosphora*	*Mytilina*	*Tripleuchlanis*
			Euchlanis	*Notholca*	
	Harpacticoids		*Filinia*	*Notommata*	*Bdelloids*

2.6. Statistical Analysis

The relationships between zooplankton metrics and environmental variables (total phosphorus, Chl-*a*, dissolved oxygen, temperature, and Secchi disk depth) were calculated using Pearson's correlation. Every correlation was performed individually and not in unison, therefore, a Bonferroni correction was not needed. Multiple regression (stepwise procedure) was performed to identify relationships between zooplankton metrics and environmental parameters (zooplankton variables entered the analysis only if $p < 0.05$). In order to test the validity of zooplankton metrics and indicate significative differences among different categories within trophic state and ecological potential, metrics' average values were compared using a *t*-test ($p < 0.05$) in each consecutive category. Additionally, the same test was conducted to compare oligotrophic vs. eutrophic, verifying the difference between low and high productive waters. The data used in the statistical analyses were previously normalized log(x + 1) to meet assumptions of homoscedasticity and normal distribution of residuals. Data analyses were performed using R 4.0.0 "Arbor day" version [56] and plots were created with the R package "ggplot2".

3. Results

3.1. Environmental Data, Trophic State, and Ecological Potential

During the present study, 304 samples were obtained. Each sample was considered as data that corresponded to the reservoir and the year sampled (for example, Mequinenza 2016). The environmental data across the basin reservoirs exhibited a wide range of values. Minimum and maximum values were as follows: chlorophyll *a*—Sopeira 2019 (0.4 μg/L) and El Val 2019 (51.8 μg/L); total phosphorus—Oliana 2012 (0.65 μg/L) and Mezalocha 2012 (186 μg/L); temperature—Llauset 2017 (10.3 °C) and Guiamets 2012 (28.1 °C); dissolved oxygen–Flix 2011 (2.5 mg/L) and El Val 2015 (14.38 mg/L); Secchi disk depth–Mezalocha 2012 (0.23 m) and Cavallers 2015 (18 m). The reservoirs' trophic states were classified according to Carlson [42]: 123 oligotrophic, 123 mesotrophic, 55 eutrophic, and 3 hypereutrophic. In the case of ecological potential, using the WFD guidance, the reservoirs were classified thusly: 99 good or superior, 202 moderate, and 3 poor; none were registered as bad. The complete data related to the trophic state and ecological potential of each reservoir can be found in Appendix A.

3.2. Zooplankton Community Description

The total number of zooplankton species identified in the current research was 169, composed mainly of rotifers (115), followed by cladocerans (36), and copepods (17). The complete zooplankton species list can be found in Appendix B Table A2. For different years, the zooplankton density and biomass of the three principal groups varied among reservoirs. The minimum abundance was presented in Flix 2012 (6.76 ind L^{-1}) and the maximum in La Sotonera 2017 (2758 ind L^{-1}). In the case of biomass estimated as dried weight (DW), a minimum was found in Peña 2013 (0.45 μg DW L^{-1}) and a maximum in Gallipuén (1971 μg DW L^{-1}). All these minimums and maximums were recorded in reservoirs with high and low water quality, respectively.

Zooplankton density and biomass averages increased with the trophic and ecological potential (Figure 2). The same pattern can be observed in large zooplankton; however, the small zooplankton did not show this strong increase pattern (Figure 3).

Concurrently, there was a decrease in the ZOO:CHLA ratio; higher ratios were found in reservoirs with high water quality and lower ratios in those with low water quality. The ZOO:PHYTO ratio did not show a strong decrease in trophic state, but in ecological potential, it presented the same pattern as the ZOO:CHLA ratio (Figure 4). Splitting the zooplankton into different groups, the rotifers did not experience large differences in levels of either trophic state or ecological potential. Meanwhile, the microcrustaceans abundance and biomass increased for higher eutrophic levels (Figure 5).

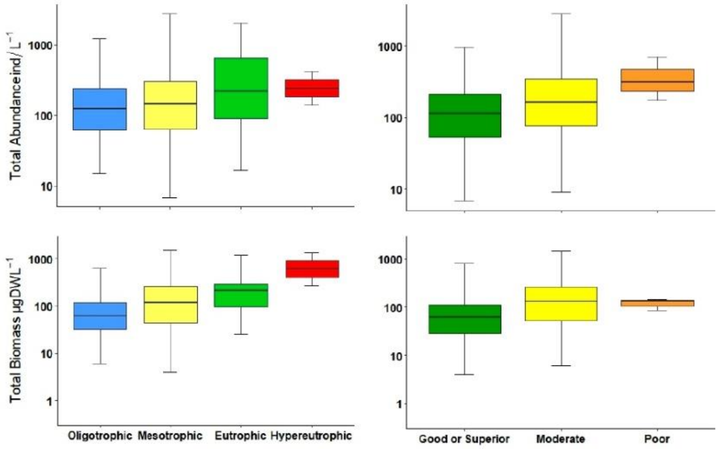

Figure 2. Boxplots of total abundance and biomass of zooplankton; (**left side**) trophic status, (**right side**) ecological potential. The box bounds the interquartile range (IQR; 25–75 percentile), the horizontal line inside the box indicates the median, and whiskers (error bars) indicate the 90th above and 10th below percentiles.

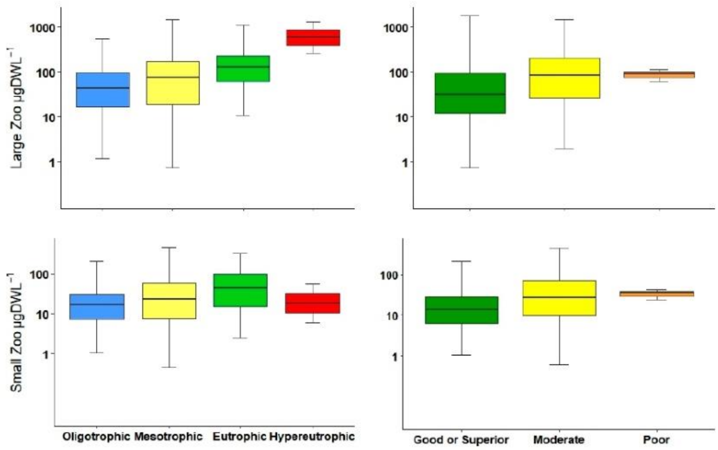

Figure 3. Boxplots of large and small zooplankton biomass; (**left side**) trophic status, (**right side**) ecological potential.

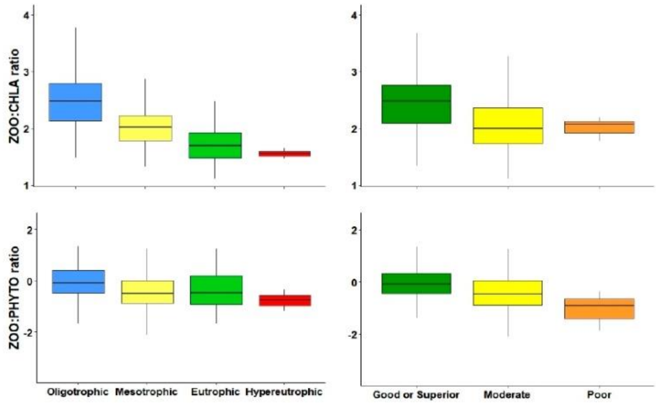

Figure 4. Boxplots of zooplankton:Chl-*a* and zooplankton:phytoplankton ratios; (**left side**) trophic status, (**right side**) ecological potential. Data was previously normalized (log(x + 1)).

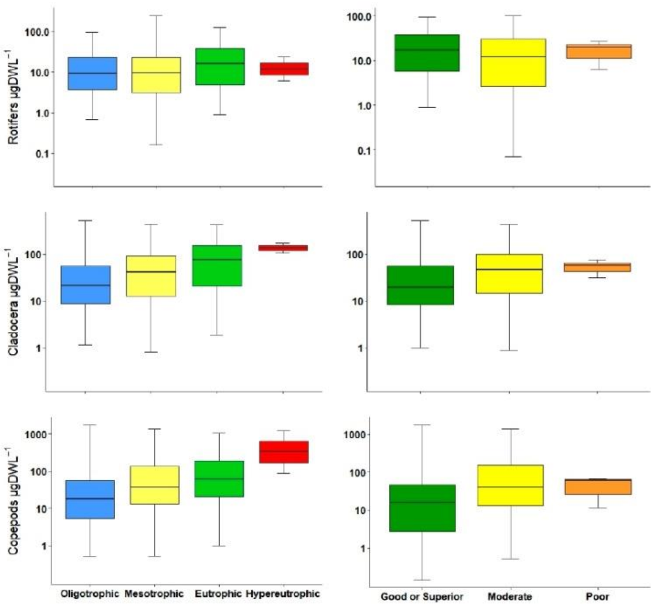

Figure 5. Zooplankton biomass of major groups; (**left side**) trophic status, (**right side**) ecological potential.

Finally, separating the last microcrustacean group into daphnids, calanoids, and cyclopoids, the copepods presented a slight increase in eutrophicated reservoirs and low-quality waters (Figure 6).

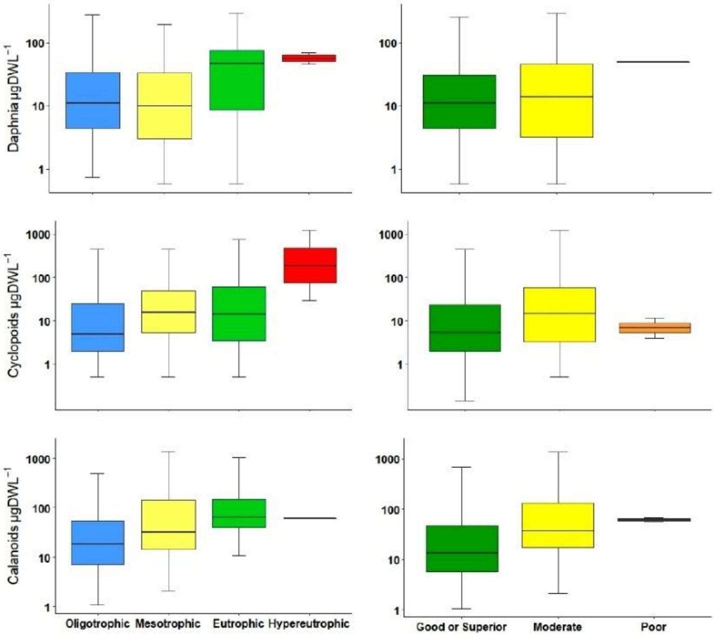

Figure 6. Zooplankton biomass divided into orders or genera, (**left side**) trophic status, (**right side**) ecological potential.

3.3. Statistical Interpretation

Pearson correlations showed that most zooplankton metrics were correlated significantly with variables that mainly determined the water quality of the reservoir (Table 2). All metrics were correlated with chlorophyll *a*, followed in number by total phosphorus, and Secchi disk. In contrast, the dissolved oxygen parameter had the lower number of significant correlations with metrics; only with copepod density and ZOO:PHYTO biomass ratio. The metrics that were correlated with only one parameter were rotifer density and *Daphnia* biomass; both were correlated with chlorophyll *a*. The strongest correlation was between the ZOO:CHLA ratio and TP and Chl-*a* variables.

Table 2. Pearson's correlations between environmental variables and zooplankton metrics. TP—total phosphorus, CHLA—chlorophyll *a*, Temp—temperature, DO—dissolved oxygen, SD—Secchi disk. ZOO—total zooplankton, LZOO—large zooplankton, SZOO—small zooplankton, ZOO:CHLA—zooplankton:chlorophyll *a* ratio, ZOO:PHYTO—zooplankton:phytoplankton ratio, ROT—rotifers, CLAD—cladocerans, COP—copepods, DAPHN—*Daphnia*, CYCLO—cyclopoids, CALA—calanoids. Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Metric	Coefficients				
	TP	**CHLA**	**Temp**	**DO**	**SD**
			Density		
ZOO	0.18 ***	0.33 ***	0.24 ***		−0.14 *
LZOO	0.26 ***	0.29 ***	0.30 ***		0.16 **
SZOO	0.14 *	0.24 ***	0.29 **		
ZOO:CHLA	−0.37 ***	−0.64 ***	−0.14 *		0.32 ***
ZOO:PHYTO		−0.33 ***	−0.22 ***		
ROT		0.18**			
CLAD	0.16 **	0.39 ***	0.36 ***		−0.16 **
COP	0.25 ***	0.28 ***	0.28 ***	−0.12 *	−0.17 **
DAPHN	0.14 *	0.19 **			
CYCLO	0.27 ***	0.20 **	0.18 **		−0.20 **
CALA	0.25 **	0.27 **	0.27 **		−0.24 **
			Biomass		
ZOO	0.24 ***	0.37 ***	0.32 ***		−0.17 **
LZOO	0.24 ***	0.26 ***	0.27 ***		0.16 **
SZOO	0.21 **	0.36 ***	0.22 ***		−0.12 *
ZOO:CHLA	−0.37 ***	0.64 ***	−0.14 *		0.32 ***
ZOO:PHYTO		−0.23 ***		−0.16 **	
ROT	0.13 *	0.23 ***			
CLAD	0.13 *	0.34 ***	0.32 ***		
COP	0.19 **	0.24 ***	0.26 ***		−0.13 *
DAPHN		0.16 *			
CYCLO	0.24 **	0.17 *	0.15 *		−0.18 **
CALA	0.26 **	0.28 **	0.27 **		−0.25 **

The multiple regressions produced through stepwise variable selection indicate that, like in the Pearson correlation, the chlorophyll *a* was the variable with a greater effect on different zooplankton metrics, being significantly correlated to all variables except for cyclopoids. Total density and biomass were positively correlated to temperature and chlorophyll *a* only. The metrics of ZOO:PHYTO ratio, density of cladocerans, cyclopoid density and biomass were significantly correlated to TP. Large zooplankton and micro-crustacean metrics had highly positive correlations, especially with temperature. On the contrary, dissolved oxygen was not significant to any metric, and Secchi disk was not correlated to anything. The analysis explained between 2% and 54% of the variability in the metrics of zooplankton (Table 3).

Table 3. Results of multiple regression analysis (stepwise procedure) between environmental variables and zooplankton metrics. Independent variables: TP—total phosphorus, CHLA—chlorophyll *a*, Temp—temperature, DO—dissolved oxygen, SD—Secchi disk. Dependent variables: zooplankton metrics. Variable names are as denoted in Table 2. Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and ns—no significant.

Variable	Coefficients					Regression Statistics			
	TP	*Chla*	DO	T	SD		*p*	r^2	*n*
					Density				
ZOO		0.40^{***}		1.04^{*}		F2 = 40.41	0.0001	0.1166	292
LZOO		0.40^{**}		2.15^{***}		F2 = 20.60	0.0001	0.122	280
SZOO		0.35^{**}	0.57^{ns}	0.91^{ns}		F3 = 7.433	0.0001	0.06	291
ZOO:CHLA		-1.11^{***}	0.45^{ns}			F2 = 178.8	0.0001	0.5474	292
ZOO:PHYTO	0.32^{**}	-0.80^{***}		-1.51^{*}		F3 = 16.46	0.0001	0.13	288
ROT		0.35^{**}				F1 = 9.88	0.0018	0.02	293
CLAD	-0.25^{*}	0.76^{***}		2.82^{***}		F3 = 27.23	0.0001	0.2112	291
DAPHN		0.36^{**}				F1 = 8.557	0.0037	0.032	227
COP		0.57^{**}	-0.91^{ns}	2.40^{**}		F3 = 13.62	0.0001	0.1141	291
CYCLO	0.46^{**}			1.16^{ns}		F2 = 9.605	0.0001	0.07	213
CALA		0.45^{**}	-0.8304^{ns}	1.74^{ns}		F3 = 6.783	0.0002	0.1043	146
					Biomass				
ZOO		0.47^{***}		1.88^{***}		F2 = 31.28	0.0001	0.1708	292
LZOO	0.18^{ns}	0.26^{ns}		1.97^{**}		F3 = 11.43	0.0001	0.0999	279
SZOO		0.61^{***}		0.98^{ns}		F2 = 24.17	0.0001	0.1361	292
ZOO:CHLA		-1.03^{***}		0.92^{*}		F2 = 108.6	0.0001	0.4226	292
ZOO:PHYTO		-0.48^{***}	-0.70^{ns}	1.27^{*}		F3 = 10.12	0.0001	0.08	288
ROT		0.42^{***}				F1 = 16.62	0.0001	0.05	293
CLAD	-0.30^{*}	0.74^{***}	-0.63^{ns}	2.31^{**}		F4 = 15.76	0.0001	0.1672	290
COP		0.53^{**}		3.47^{**}		F2 = 15.07	0.0001	0.08	292
DAPHN		0.29^{*}				F1 = 5.67	0.0189	0.02	227
CYCLO	0.48^{**}					F1 = 13.45	0.0003	0.054	214
CALA		0.46^{**}	-0.8304^{ns}	1.80^{ns}		F2 = 6.972	0.0002	0.1073	146

According to *t*-test analysis, several metric means were statistically different in consecutive categories of trophic status and the levels that separate high water quality from low water quality in their ecological potential. Metric significance varied between consecutive levels; some were significant between oligo–mesotrophic and others in meso–eutrophic. However, none of the metrics were statistically different between eutrophic and hypereutrophic ($p > 0.05$); this was probably due to the low number of samples classified as hypereutrophic. Large zooplankton density and the ZOO:CHLA biomass ratio were significant between moderate and poor ($p < 0.05$); the rest of the metrics did not show any significance among these levels. Additionally, most of the metrics were statistically different between oligo–eutrophic and good–moderate ($p < 0.05$), the only metrics that were not significant among these levels were small zooplankton density and the ZOO:PHYTO biomass ratio (Table 4).

Table 4. *t*-Student test between zooplankton metrics, trophic status, and ecological potential levels. (Oligo—oligotrophic, Meso—mesotrophic, Eutro—eutrophic, Hyper—hypereutrophic, Good—Good or Superior). Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Metric	Trophic Status				Ecological Potential	
	Oligo–Meso	Meso–Eutro	Eutro–Hyper	Oligo–Eutro	Good–Moderate	Moderate–Poor
				Density		
ZOO		*		**	***	
LZOO		**		***	***	**
SZOO					*	
ZOO:CHLA	***	***		***	***	
ZOO:PHYTO	***			*	***	
ROT						
CLAD		*	**	***	***	
DAPHN		**	*	*		
COP	**			**	***	
CYCLO	***			***	***	
CALA	*			**	**	
				Biomass		
ZOO	*	**		***	***	
LZOO		*		***	***	
SZOO		*		***	***	
ZOO:CHLA	***	**		***	***	***
ZOO:PHYTO						
ROT					*	
CLAD		*	*	***	**	
DAPHN		*	*	*		
COP	*			***	***	
CYCLO	***			*	**	
CALA	*			**	**	

4. Discussion

The current study presents data from reservoirs widely distributed at the Ebro watershed, located in the Mediterranean area. It presents high variation of environmental data (most of the different trophic states and ecological potentials were present) indicating the high heterogeneity across the sampled reservoirs. Through the statistical treatment applied in the present research to the large dataset, we were able to define the zooplankton metrics that are related to environmental conditions and could be used to determine trophic status and ecological potential.

Several environmental variables are of a high importance in determining the water quality in lentic waters. One of these is the concentration of TP (a classical bottom-up variable). TP was correlated with a high number of the zooplankton metrics that were proposed in the current study. However, the chlorophyll *a* was the variable most correlated with metrics, in contrast with dissolved oxygen, which was not related with many metrics. Nevertheless, not only did environmental data vary, zooplankton metrics also showed different patterns through reservoirs and trophic gradients.

The total abundance of zooplankton as indicator of trophic state has been tested in another Iberian watershed [38], and as a water quality tool in natural Mediterranean lakes [36], both exhibiting an increment in zooplankton density along with both eutrophication and ecological potential increase, similarly to our results. Although biomass is dependent on abundance, it is an interesting metric to consider, because the change in any group dominance can mean high changes in the biomass and an increment in the percentage of microcrustaceans in relation to rotifers and zooplankton biomass has been linked to TP in different climatic zones [54]. Biomass increased along the trophic gradient but showed a strong pattern when compared to abundances, as observed in European lakes [33,36]. Both density as well as biomass were statistically significant in differentiating between almost all water quality classes; however, biomass was slightly better to separate oligotrophic from mesotrophic environments. Nevertheless, both metrics, abundance and biomass could be used to determine bad or good water quality.

Normally, most of the total biomass is comprised of large-bodied zooplankton (mainly microcrustaceans), and thus, large-bodied biomass is very similar to total biomass. This is unlike total abundance, which may differ in the presence of small-bodied zooplankton. Gyllström et al. [54] reported a positive relationship between the increment of large zooplankton biomass and TP. In the current study, we found a similar tendency along the trophic gradient and ecological potential. Additionally, the density of the large zooplankton presented similar patterns and could distinguish marginally better between ecological potentials than biomasses.

Small-bodied zooplankton, mainly composed of rotifers and small cladocerans, were correlated with variables that determined the trophic status, particularly with Chl-*a*. These small zooplankton can shape the community in terms of abundance, especially for the high number of rotifer individuals [10,22]. These metrics were effective in distinguishing between low and high productive waters in general, but less sensible than other metrics in differentiating precisely between consecutive levels. A high elevate abundance and biomass could indicate an increment in the trophic gradient or other environmental pressures [54].

An important aspect related to the zooplankton community is the high pressure from planktivorous fish [57], which strongly affect zooplankton abundance, biomass, community composition, and even the size of the structure, especially in low productive waters [58–60]. On the other hand, phytoplankton blooms, especially cyanobacterial blooms, can become an additional pressure [61]. Cyanobacteria are a poor-quality food resource [62], produce harmful cyanotoxins [63,64], and can promote zooplankton community shift into small-bodied species [65–67]. In comparison to large-bodied species, small species are less mechanically affected (clogging of their filtering apparatus) by the presence of cyanobacterial colonies, mainly of filamentous forms [68] in habitats with a eutrophic increment, where such blooms normally surge [59]. Hence, the use of large and small zooplankton as a metric could provide valuable data about both fish and cyanobacterial pressures.

The crustacean index used in Polish lakes is the NCRU [32]; however, it is inclusive of all crustaceans in general. In our study, in order to detect possible variations, we decided to divide them into groups. In our results, calanoid copepod abundances and biomass increased marginally from oligotrophic to eutrophic systems, but normally, at high trophic status, calanoids decreased; for this reason, they are typically considered to be from oligotrophic waters [25,55]. Cyclopoids showed a considerable increment, especially inside eutrophic and hypereutrophic reservoirs; lately, their presence and the occurrence of *Acanthocyclops* species are used as indicators of eutrophic conditions in Mediterranean waterbodies [26,27,29,34]. For this study in general, the use of copepod metrics, including both calanoids and cyclopoids, resulted in the same correlation between environmental variables, and was a reliable indicator to differentiate between low and high productive waters. However, these metrics combined with the use of an indicator species could lead to a better water quality classification.

The Cladocera and daphnids metrics were very similar in that they were both effective differentiating among the trophic status levels; however, in ecological potential, only the Cladocera metric had the ability to detect between good and moderate levels. This lack of *Daphnia* sensitivity was probably because most reservoirs were classified as both good or superior and moderate, and had a wide value range in environmental variables such as TP and Chl-*a*. Another reason for the differences between metrics could be the presence or absence of *Diaphanosoma* spp. and *Holopedium* spp. as the dominant Cladocera inside reservoirs.

In several European waterbodies, there was a positive relationship between TP concentration and *Daphnia* spp. in different waterbodies; at higher TP concentration a biomass increase has been reported [54,59]. This is in accordance with our results: in lower trophic levels, daphnids abundance and biomass were lower, and there was an increase at higher levels. Furthermore, it is important to point out that within this group, there is a species that considered to be an indicator for both low (*Daphnia longispina*) and high eutrophic levels (*Daphnia cucullata*) [26,27,37]. These species were present only in reservoirs with low and high trophic status, respectively.

In eutrophic reservoirs, an increase in rotifers was detected in comparison with those of oligotrophic status [69]. These findings were in concurrence with our results; however, rotifer metrics had the lesser correlation with environmental variables, and they were not sensitive enough to indicate separation in trophic status or ecological levels compared to other metrics. Nevertheless, in natural lakes, this group is a useful tool to determine the trophic status using abundances [30,31] or biomass [31,33]. Moreover, not only biomass and abundances are related to trophic gradient, but several studies in natural and man-made waterbodies have pointed to the rotifer species as being good indicators of trophic status and water quality [22,26,27,29,35,37,70]; therefore, this group can provide valuable data and should not be overlooked.

An indicator of the cascading effects that zooplankton have on phytoplankton is the ZOO:PHYTO ratio [8]. Previous studies in Danish lakes [55,59] and shallow sub-tropical lakes [71] indicate that this ratio decrease coincides with an increase in TP. Here, we recorded a slight downward trend in this ratio along TP increase, although, we did not find a significant correlation; this is in accordance with results obtained in the Jucar watershed [38]. These previous studies used the ZOO:PHYTO biomass, but, in our results, the use of biomass was not capable of differentiating between levels in trophic and ecological potential, whereas, the use of abundances could detect between low and high productive reservoirs.

A similar metric is the ZOO:CHLA ratio, using the measurement of phytoplankton production. In our study, the ZOO:CHLA ratio was very sensitive to changes in trophic gradient and, along with ZOO and CLAD, was the most effective metric differentiating most levels of both trophic state and ecological potential. They were also closely related to variables that determine water quality. An advantage of the ZOO:CHLA ratio is that it is not imperative that one be an expert zooplankton taxonomist, since it is only necessary to identify and count the major groups in general without reaching the species level, and Chl-*a* data can be obtained through any method to apply this metric.

The decrease in both ZOO:CHLA and ZOO:PHYTO metrics along the increase in the trophic gradient could be explained by bottom-up effect [72]; the abundance and biomass of phytoplankton can change due to several variables such as cyanobacterial blooms, typically during summertime in productive waters with high levels of TP, and the replacement of edible phytoplankton with inedible and low quality species. Fish exert an additional pressure on zooplankton, especially in warmer waters [54].

Zooplankton metrics such as density, biomass, large body size, cladocerans, and the ZOO:CHLA ratio can be used as good overall indicators to differentiate between trophic state and ecological potential levels. Metrics related to copepods, as well as their division in their orders, calanoids and cyclopoids, are especially sensible for distinguishing reservoirs with better water quality (such as oligotrophic from mesotrophic), trophic state, and good or higher from moderate in ecological potential.

The aim of the WFD was to bring European waterbodies up to good ecological levels. According to current research, most zooplankton metrics have been shown to be good indicators to differentiate between reservoirs that have good ecological potential and others. The integrative capacity of zooplankton with the environmental factors that determine the trophic state and the ecological potential, can give us a broader picture over time compared to phytoplankton. Due to their shorter lifespan, as well as their community composition, phytoplankton can change in a short time compared to zooplankton [73], and even under specific environmental pressures or blooms, the phytoplankton could not give data as accurate. Thus, the use of the different zooplankton metrics presented here, along with indicator species, can be used as a tool to determine the water's quality. Zooplankton collection does not present a great impact on budget nor working time because it can be sampled in parallel with phytoplankton. Phytoplankton sampling is included in monitoring programs, hence, the use of both plankton components is reasonable, in addition to being complementary, and could give us more precise water quality information. Finally, as several authors have recently reported, zooplankton can be a good indicator to determine both trophic status and ecological potential. Therefore, we strongly recommend that zooplankton be incorporated as one more BQE within the Water Framework Directive.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/w13172382/s1, Table S1: Average physical and chemical data from reservoirs of the Ebro Watershed. Samples = number of years that a reservoir was sampled, Depth = reservoir maximum sampling depth, Volume = reservoir maximum volume, T = temperature, DO = dissolved oxygen, SD = Secchi disk depth, SS = duspend solids, Chl-*a* = chlorophyll *a*, TP = total phosphorus.

Author Contributions: Writing—Editing, M.E.M.-C., J.M.S. and E.V.; Conceptualization, methodology, E.V., J.M.S., X.S.-P. and M.D.S.; Investigation, M.E.M.-C., E.V. and J.M.S.; Field Work, X.S.-P., M.D.S., E.V., J.M.S. and M.E.M.-C.; Formal Analysis, M.E.M.-C. All authors have read and agreed to the published version of the manuscript.

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Appendix A

Table A1. Reservoir code, name, trophic status, ecological potential and location.

Code	Reservoir	Average Trophic Status	Average Ecological Potential	Location
ALB	Albiña	Oligo–Mesotrophic	Moderate	Pais Vasco
ALL	Alloz	Oligotrophic	Good–Moderate	Navarra
ARD	Ardisa	Eutrophic	Moderate	Aragón
BAL	Balaguer	Mesotrophic	Good or superior	Cataluña
BAR	Barasona	Oligotrophic	Good or superior	Aragón
BAS	Baserca	Oligotrophic	Good or superior	Aragón
BUB	Búbal	Oligotrophic	Good or superior	Aragón
CAL	Calanda	Oligotrophic	Good–Moderate	Aragón
CAM	Camarasa	Oligotrophic	Good or superior	Cataluña
CAN	Canelles	Oligotrophic	Good or superior	Aragón
CAS	Caspe	Mesotrophic	Moderate	Aragón
CAV	Cavallers	Oligotrophic	Good or superior	Cataluña
CER	Cereceda	Eutrophic	Moderate	Castilla y León
CIU	Çiurana	Oligotrophic	Good or superior	Cataluña
COR	El Cortijo	Eutrophic	Moderate	La Rioja
CUE	Foradada	Mesotrophic	Moderate	Aragón
EBR	Ebro	Oligo–Mesotrophic	Moderate	Cantabria
ESC	Escales	Oligotrophic	Good or superior	Aragón

Table A1. *Cont.*

Code	Reservoir	Average Trophic Status	Average Ecological Potential	Location
ESR	Escarra	Oligotrophic	Good or superior	Aragón
EST	Alcañiz	Mesotrophic	Good–Moderate	Aragón
EUG	Eugui	Oligotrophic	Good or superior	Navarra
FLI	Flix	Mesotrophic	Moderate	Cataluña
GAL	Gallipuén	Mesotrophic	Moderate	Aragón
GRA	El Grado	Oligotrophic	Good or superior	Aragón
GUI	Guiamets	Mesotrophic	Moderate	Cataluña
IRA	Irabia	Oligotrophic	Moderate	Navarra
ITO	Itoiz	Oligotrophic	Good or superior	Navarra
LAN	Lanuza	Oligotrophic	Good or superior	Aragón
LEC	Lechago	Oligo–Mesotrophic	Moderate	Aragón
LLA	Llauset	Oligotrophic	Good or superior	Aragón
LOT	La Loteta	Meso–Eutrophic	Moderate	Aragón
MAE	Maidevera	Mesotrophic	Moderate	Aragón
MAN	Mansilla	Oligotrophic	Good–Moderate	La Rioja
MAR	Margalef	Mesotrophic	Moderate	Cataluña
MED	Mediano	Oligotrophic	Good or superior	Aragón
MEQ	Mequinenza	Oligo–Mesotrophic	Moderate	Aragón
MEZ	Mezalocha	Meso–Eutrophic	Moderate	Aragón
MOA	Montearagon	Oligotrophic	Good–Moderate	Aragón
MON	Vicarías	Mesotrophic	Moderate	Castilla y León
MOV	Moneva	Meso–Eutrophic	Moderate	Aragón
OLI	Oliana	Mesotrophic	Moderate	Cataluña
ORT	Ortigosa	Oligotrophic	Good or superior	La Rioja
PAJ	Pajares	Oligotrophic	Good or superior	La Rioja
PEÑ	La Peña	Mesotrophic	Moderate	Aragón
PEN	Pena	Oligotrophic	Good or superior	Aragón
PUE	Puentelarra	Mesotrophic	Moderate	Castilla y León
RIA	Rialb	Mesotrophic	Moderate	Cataluña
RIB	Ribarroja	Eutrophic	Moderate	Cataluña
SAB	Sabiñanigo	Oligotrophic	Good or superior	Aragón
SAN	Santa Ana	Oligotrophic	Good or superior	Cataluña
SLO	San Lorenzo	Mesotrophic	Good or superior	Cataluña
SOB	Sobrón	Meso–Eutrophic	Moderate	Castilla y León
SOP	Sopeira	Oligotrophic	Good or superior	Aragón
SOT	Sotonera	Mesotrophic	Moderate	Aragón
STO	Santolea	Oligotrophic	Good or superior	Aragón
TAL	Talarn	Oligo–Mesotrophic	Good or superior	Cataluña
TER	Terradets	Mesotrophic	Moderate	Cataluña
TOR	Las Torcas	Oligo–Mesotrophic	Good or superior	Aragón
TRA	Tranquera	Mesotrophic	Moderate	Aragón
ULL	Ullivari	Oligo–Mesotrophic	Good–Moderate	Pais Vasco
URD	Urdalur	Oligotrophic	Good or superior	Navarra
URR	Urrunaga	Oligo–Mesotrophic	Moderate	Pais Vasco
UTC	Utexa seca	Eutrophic	Moderate	Cataluña
VAD	Vadiello	Oligo–Mesotrophic	Good or superior	Aragón
VAL	Val	Eutrophic	Moderate	Aragón
YES	Yesa	Oligotrophic	Good–Moderate	Navarra

Appendix B

Table A2. Zooplankton species present in the Ebro watershed.

Rotifera			
Class Bdelloidea	*C. unicornis*	*L. puriformis*	*P. triloba*
Bdelloids	*Conochilus* sp.	*L. stenroosi*	*Proales* sp.
Class Monogononta	*Dicranophorus* sp.	*L. stichaea*	*Ptygura* sp.
Anuraeopsis fissa	*Encentrum* sp.	*L. tenuiseta*	*Squatinella rostrum*
Ascomorpha ecaudis	*Eosphora* sp.	*Lecane* sp.	*Synchaeta grandis*
A. ovalis	*Euchlanis dilatata*	*Lepadella acuminata*	*S. kitina*
A. saltans	*Filinia longiseta*	*L. ovalis*	*S. longipes*
Ascomorpha sp.	*F. terminalis*	*L. patella*	*S. oblonga*
Asplanchna girodi	*Gastropus stylifer*	*L. rhomboides*	*S. pectinata*
A. priodonta	*Hexarthra fennica*	*Lophocaris salpina*	*S. stylata*
A. sieboldi	*H. intermedia*	*L. oxysternon*	*S. tremula*
Asplanchna sp.	*H. mira*	*Macrochaetus subquadratus*	*Synchaeta* sp.
Brachionus angularis	*H. oxyuris*	*Monommata appendiculata*	*Testudinella incisa*
B. bidentata	*Hexarthra* sp.	*Mytilina mucronata*	*T. mucronata*
B. calyciflorus	*Kellicottia longispina*	*Notholca acuminata*	*T. patina*
B. dimidiatus	*Keratella cochlearis*	*N. squamula*	*Trichocerca cylindrica*
B. havanaensis	*K. cochlearis tecta*	*Notommata allantois*	*T. gracilis*
B. plicatilis	*K. hiemalis*	*N. copeus*	*T. inermis*
B. quadridentatus	*K. quadrata*	*Ploesoma hudsoni*	*T. insignis*
B. urceolaris	*K. tropica*	*P. lenticulare*	*T. pusilla*
Cephalodella gibba	*Lecane aculeata*	*P. truncatum*	*T. similis*
C. stenroosi	*L. bulla*	*Polyarthra dolichoptera*	*T. tenuinor*
Cephalodella sp.	*L. clara*	*P. euryptera*	*T. tigris*
Collotheca pelagica	*L. closterocerca*	*P. longiremis*	*Trichocerca* sp.
Collotheca sp.	*L. cornuta*	*P. luminosa*	*Trichotria pocillum*
Colurella colurus	*L. flexilis*	*P. major*	*T. tetractis*
C. obtusa	*L. furcata*	*P. minor*	*Tripleuchlanis plicata*
C. uncinata	*L. inermis*	*Polyarthra vulgaris*	
Conochilus dossuarius	*L. luna*	*Polyarthra* sp.	
C. natans	*L. lunaris*	*Phompolyx sulcata*	
Crustacea			
Suborder Cladocera			
Alona affinis	*D. parvula*	**Copepoda**	**Order Harpacticoida**
A. guttata	*D. pulicaria*	**Order Cyclopoida**	Harpacticoids
A. quadrangularis	*Daphnia rosea*	*Acanthocyclops americanus*	
A. rectangula	*Diaphanosoma brachyurum*	*A. robustus*	**Order Poecilostomatoida**
Alona sp.	*D. lacustris*	*Cyclops abyssorum*	*Ergasilus sieboldi*
Alonella exigua	*D. mongolianum*	*C. lacustris*	*Neoergasilus japonicus*
A. nana	*Diaphanosoma* sp.	*C. vicinus*	
Bosmina longirostris	*Holopedium gibberum*	*Cyclops* sp.	**Mollusca**
Ceriodaphnia dubia	*Ilyocryptus sordidus*	*Eucyclops serrulatus*	**Class Bivalvia**
C. laticaudata	*Leydigia acanthocercoides*	*Eucyclops* sp.	*Dreissena polymorpha*
C. pulchella	*L. leydigi*	*Macrocyclops albidus*	
C. quadrangula	*L. quadrangularis*	*Thermocyclops dybowskii*	
Chydorus sphaericus	*Macrothrix hirsuticornis*	*Tropocyclops prasinus*	
Daphnia cucullata	*M. laticornis*		
D. curvirostris	*Moina micrura*	**Order Calanoida**	
D. galeata	*Oxyurella tenuicaudis*	*Copidodiaptomus numidicus*	
D. longispina	*Phrixura leei*	*Eudiaptomus vulgaris*	
D. magna	*Sida crystalina*	*Neolovenula alluaudi*	

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Chapter 4

Zooplankton functional groups and machine learning to determine water quality in reservoirs.

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Abstract

The present research is the first to use a zooplankton functional-trait and machine learning approach to determine reservoirs´ ecological potential and trophic status and presents a suggestion to include zooplankton as indicator within the European Union Water Framework Directive. Here, using more than 300 samples obtained from reservoirs during ten years in a large Mediterranean basin, we determined which zooplankton functional groups (ZFG) are present. Then, we investigated the environmental variables that were related to them. Five functional groups were obtained: large filter copepods, raptorial copepods, cladocerans, microphagous rotifers and raptorial rotifers. The physicochemical variables that were related to ZFG were mainly chlorophyll-a, total phosphorus, temperature, and Secchi disk. The functional approach indicated that in general microcrustaceans were better indicators than rotifers; low densities of large filter-feeding groups like calanoid copepods and cladocerans were indicators of high-water quality (oligotrophy and good or higher ecological potential). Meanwhile, high cyclopoid copepods abundances served as indicators of low water quality (eutrophy and moderate-poor ecological potential). Using a machine learning approach, Random Forest predicts models' accuracy varied from 41% to 77% among functional groups and water quality levels. Finally, Classification and Regression Trees were used to estimate the thresholds among categories using ZFG abundances. For ecological potential, thresholds to differentiate good or higher status from moderate were: calanoids <1.4 ind. L^{-1}, cyclopoids <0.38 ind. L^{-1} and cladocerans <19.36 ind. L^{-1}. While for trophic status between oligotrophic and eutrophic conditions were:

calanoids <3.1 ind. L^{-1}, cyclopoids <0.76 ind. L^{-1} and cladocerans <19.04 ind. L^{-1}.

Keywords: Trophic status, ecological potential, microcrustaceans, reservoirs, Random Forest, bioindicators.

Introduction

Freshwater bodies are a vital resource for diverse ecosystems and for human development. These aquatic systems are inhabited by a high number of different species. Currently, due to accelerated eutrophication caused by the impact of human activities or cultural eutrophication (Schindler 2012), many water bodies have decreased their water quality. This situation is mainly due to the increment of nutrients (phosphorus and nitrogen) along the water column, which leads to an increase in phytoplankton biomass, emergence of cyanobacterial blooms, species turnover and changes in the water quality. These, together with climate change, may threaten the water resources (Lewis et al. 2011, Moss 2011).

In response to the situation that aquatic resources are facing, different strategies have been implemented around the world. In the European Union, the European Water Framework Directive (WFD) was developed (Directive 2000). This directive establishes the measurement procedures and categories in which the different water bodies should be classified. Also, it strives to have their waterbodies in a "good quality" status throughout the medium and long-term. Since the release of this directive, there has been a great surprise and controversy arisen from the fact that the only Biological Quality Element (BQE) for planktonic organisms that was included was the phytoplankton, leaving aside the zooplankton and the information that this community could potentially provide (Caroni and Irvine 2010, Jeppesen et al. 2011).

Zooplankton communities have a fundamental ecological function in aquatic food webs (Kiørboe 2008, Haberman and Haldna 2014), contributing significantly to nutrient recycling and being the connector in the energy transfer between primary producers such as phytoplankton and higher consumers such as fish (Lampert and Sommer 1997, Wetzel 2001, Leoni 2016). Furthermore, zooplankton can shape the phytoplankton abundance and composition by applying pressure in the top-down control or respond quickly to bottom-up process (Carpenter et al. 2001, Naselli-Flores and Rossetti 2010). In the same way as algae, the zooplankton community is affected by environmental variables, such as water chemistry (pH, dissolved oxygen, nutrients, conductivity, temperature, etc.), physical (water body morphology, water streams, water mixture) and biotic factors (food availability, competitors and predators´ presence or absence) (Dodson et al. 2009). Moreover, due to cascading trophic effects, zooplankton can respond quickly to changes in the water quality than phytoplankton (Pace et al. 2013). Thus, zooplankton present characteristics to be a water quality indicator (Anas et al. 2013).

Within the zooplankton studies, there is a good representation of biogeography, ecotoxicology, ecology and physiology research (Litchman et al. 2013), however, compared to these, the number of studies based on functional groups or traits is still low. The functional groups are those species that have the same characteristic or traits and perform same roles or functions within a determinate ecosystem and may not be taxonomically related (Steneck 2001, Reynolds et al. 2002, Blondel 2003). Within zooplankton, traits can be used depending on their ecological function and character type, most of these traits are classified into morphological, physiological, behavioral, feeding strategies, trophic position in the environment, growth, form of reproduction, survival, life history and habitat among others (Barnett et al. 2007, Kiørboe 2011, Litchman et al. 2013).

A study review performed on zooplankton functional groups-traits approach made by Fernandes et al. (2019) found that most research made on this topic was concentrated in just a few countries, specifically Canada, The USA and Brazil. Also, more than 70% of the studies were on lakes, the rest of works are shared among the remaining aquatic systems. Furthermore, it´s important to point out the scarcity of studies that use this approach as bioindicator of water quality. Therefore, there currently exists an important gap and lack of information throughout many countries and waterbodies, which should be addressed by including representative data from different regions and habitats such as The Iberian Peninsula and man-made waterbodies like reservoirs.

The present research is the first study in Spain that uses the zooplankton functional groups (ZFG) as bioindicators of water quality. This is also the first study performed using machine learning to predict reservoirs´ trophic state and ecological potential. It contributes to the knowledge of zooplankton as a tool for water resource management. The aims of this research were: I) based on selected functional traits, determine the zooplankton functional groups in the Ebro basin, II) determine the physiochemical variables related to functional groups, III) determine the trophic status and ecological potential using the ZFG with a supervised machine learning approach, IV) establish the thresholds to differentiate the categories within trophic status and ecological potential using the ZFG data. For these goals, we used a robust dataset that involved more than 300 samplings events from more than 60 reservoirs along all basin over ten years.

Material and methods

Study site

Originating in the northern part of Iberian Peninsula, the Ebro River is the largest river in Spain. It flows southeast until connecting to the Mediterranean Sea. The river has a watershed area of 86,000 km^2, which makes it one of the largest watersheds in all Mediterranean area. The data in the present research were obtained through samples from 66 reservoirs located all along the Ebro watershed (Fig. 1), through sampling campaigns during summertime from 2010 to 2019. Due to logistical and budget issues, not all reservoirs were sampled each year, however, a sample was obtained at least every 3-4 years for each reservoir. Inside each reservoir, a sampled point was established to measure environmental data, collect water, and sample phytoplankton and zooplankton. This point was set up in the deepest part, 300-500 meters away from the dam wall.

Environmental and morphologic variables

At every reservoir in the sampling point, several variables were measured *in situ* along a vertical profile: dissolved oxygen, pH, conductivity, turbidity, temperature, suspended solids and chlorophyll-a, using a Sea-Bird 19 plus V2 multisensory device (Sea Bird Electronics Inc., Bellevue, WA, USA). The following morphologic variables were obtained for each reservoir: depth, reservoir volume and percentage of reservoir volume. The water transparency was determined through the Secchi disk depth (SD). For *ex-situ* analysis and phytoplankton analysis, an integrative water sample was collected from the photic zone of each reservoir using a 25 mm inner diameter ballasted PET tube. The photic zone depth was calculated by means of light penetration using a Li-Cor quantum meter (LI-COR Environmental, Lincoln, NE, USA). When the photic zone was less than 6 m deep, an integrative water sample was collected from the water's surface down to said depth or to the bottom (Vicente et al. 2005). Also, we used a standard methodology to estimate the following variables: chlorophyll a, total phosphorus (TP), total nitrogen (TN), nitrates, ammonium, alkalinity, suspended solids and turbidity (Shoaf and Lium 1976, APHA 1998).

Reservoirs´ trophic state and ecological potential

To determine the trophic state of each reservoir we used the Trophic State Index (TSI´) (Carlson 1977). A final trophic state was defined using the average of the three variables of TSI´ (total phosphorus, chlorophyll-a and Secchi disk depth).

To obtain the ecological potential (EP) we followed the methodology in "Spanish Legislation RD 817/2015" and Directive (2000). This directive uses biological and/or physicochemical indicators to obtain the ecological potential and in the present research both indicators were assessed for a better characterization. The biological indices were obtained from four algal variables measured from phytoplankton samples (chlorophyll a, biovolume, percentage of cyanobacteria and the Index of Algae Groups (IGA) (Catalan and Ventura 2003). Based on these, the classification scheme was Good or higher, Moderate, Poor, and Bad. The physicochemical indicator was obtained from the Secchi disk depth, hypolimnetic oxygen concentration and total phosphorus. Their respective classifications were Very Good, Good, Moderate, Poor, and Bad. To establish the representative classification of each biological and physicochemical indicator, we selected the average value of the algae and physicochemical variables. Following the WFD procedure using the "one-out, all-out" rule, the worst value between both indicators was selected as the ecological potential. A detailed methodology to obtain the ecological potential can be found in CHE (2016).

Zooplankton samples

The zooplankton samples were collected using a vertical Ruttner bottle with capacity of 2.7 L. In each reservoir, we took two Ruttner bottles to obtain a total of 5.4 liters of water sample, afterwards it was filtered through a 30 µm mesh size Nytal. The depth at which the samples were collected was established at the beginning of oxycline in each waterbody, which has been reported as the richest zone of zooplankton fauna during the daylight hours (Miracle and Vicente 1983). In addition, a zooplankton vertical tow net of 45 µm mesh size Nytal was towed from 30 m depth or from the reservoir bottom until the surface, these samples were obtained mainly for taxonomic purposes. Both vertical and Ruttner bottle samples were fixed with formalin at 4% final concentration and stored in hermetic glass vials.

The zooplankton species were identified using Alonso (1996) and Błędzki and Rybak (2016) for cladocerans and copepods and Koste (1978), Nogrady et al. (1995) and Nogrady and Segers (2002) for rotifers. The samples obtained from Ruttner samples, were counted using a 20 mL sedimentation chamber under an inverted microscope (Nikon Eclipse Ti-U, objective lens 4–60x), with differential interference contrast (DIC) to obtain the corresponding specific richness and species abundance.

Zooplankton selected traits and functional groups

In order to obtain the functional groups of zooplankton, we used the following five functional traits: adult dry bodyweight (bodyweight was estimated for each taxon using 30 measured specimens using biovolume and length–weight relationships (Ruttner-Kolisko 1977, Dumont et al. 1975, Culver et al. 1985)), habitat (littoral or pelagic), trophic group (herbivorous, omnivorous, carnivorous or detritivore), reproduction form (sexual or asexual) and feeding type. In the case for this last functional trait, because every group presents different strategies, they were classified as follows: Rotifers were classified based on their genus and trophi as raptorial or microphagous (Obertegger et al. 2011). For copepods, they were classified as suspension-feeders or raptorial and finally, for Cladocera species they were classified depending on the filtration type of the families presented (B—Bosminidae, D—Daphniidae, S—Sididae, and I—Ilyocriptidae) (Barnett et al. 2007, Barnett and Beinser 2007).

With the previous information we created a matrix with numerical and categorical data from the species and their corresponding functional traits. Then a dissimilarity matrix was created using Gower´s distance with the FD package (Laliberté and Legendre 2010, de Bello et al. 2021). To assign species into groups, a hierarchical clustering analysis using the Ward method was applied to the dissimilarity matrix. The functional groups were assigned using only the data from the most dominant species of zooplankton, which are species that made up > 0.1% of the total zooplankton individuals (Table 1).

Statistical analysis and influence of environmental variables in functional groups

Previous to any statistical analysis, all environmental variables were subject to a variation inflation factor analysis (VIF) and those with high collinearity were removed from any further analysis. This was conducted with the vifstep() function in the usdm package (Naimi et al. 2014). A Principal Component Analysis (PCA) was performed to visualize the environmental variables and the water quality categories along the basin. Then, a Pearson's correlation was performed to verify lineal relationships between the ZFG numerical abundance and selected environmental variables (TP, chlorophyll a, temperature, dissolved oxygen and Secchi disk depth). In this case, each correlation was performed individually and not in unison, therefore, a Bonferroni correction was not needed. A redundancy analysis (RDA) was also performed to investigate the correlations between a forward-selected environmental and reservoir´s morphologic variables with a Hellinger transformed functional groups abundance data. For the above analyses, the environmental data was previously normalized and transformed logarithmically Log (x+1).

Determining trophic state and ecological potential through machine learning

Using a non-normalized matrix of the ZFG abundance and reservoirs physicochemical variables, we selected a Random Forest (RF) as a machine learning model to predict the water quality of reservoirs. This RF is an algorithm that generates many de-correlated decision trees and provides a robust and powerful technique to analyze ecological data (Cutler et al. 2007). For this method, two datasets were used, one with the complete number of reservoirs (full dataset), and a second (reduced dataset) using only the reservoirs classified as oligotrophic and eutrophic. We used this approach since significant differences were detected among oligotrophy and eutrophy conditions in the Ebro watershed (Muñoz-Colmenares et al. 2021a). So, the mixed data from mesotrophic reservoirs was removed for a better delimitation to establish the thresholds among the rest of the categories.

First, as screening, we ran two RFs to determine and establish which functional groups were more important in determining trophic status and ecological potential. To evaluate the importance of the selected groups we used the package permimp (Debeer et al. 2021). Then, we built RFs models to predict the different classification levels of both trophic status and ecological potential. Those models were performed by splitting the data into training dataset (80% of the data selected randomly) and the validation data (20% of the data). The RFs models were created using conditional inference trees from the party package (Hothorn et al. 2021).

Finally, to establish the thresholds among each trophic state and ecological potential, we used Classification and Regression Trees (CARTs) with the abundance of the most important ZFG from the screening RF. The CARTs were also tested with the full and reduced datasets to verify whether or not differences were detected. The package mvpart was used to construct the regression trees (Therneau and Atkinson 2014) with the total number of reservoirs as cross-validation groups of each dataset and 100 iterations. All analyses were performed using the R programming language, version 4.0.5 “Shake and Throw” (R Core team 2021).

Results

Environmental data, trophic state, and ecological potential

During the present research, a total of 66 reservoirs were sampled and we obtained 304 corresponding samples throughout the years. Each sample was considered data that corresponded to the reservoir and the year sampled (for example, Oliana 2016). Along the reservoirs in the watershed, the physicochemical parameters had a wide variation. Minimum and maximum values of some of them were: chlorophyll-*a* (0.4-51.8 μgL^{-1}), total phosphorus (0.65-186.00μgL^{-1}), temperature (10.3-28.1°C), dissolved oxygen (2.50-14.38 mgL^{-1}), Secchi disk depth (0.23–18.00 m). The trophic state of the reservoirs´ samples classified according to Trophic State Index (Carlson 1977) were 123 as oligotrophic, 123 mesotrophic, 55 eutrophic and only 3 as hypereutrophic. In the case of the ecological potential, following the WFD guidance were classified as such: 99 reservoirs´ samples as Good or higher, 202 Moderate and only 3 as Poor, none was registered as Bad. A complete information related with specific reservoir’s trophic state, ecological potential and location can be found in Muñoz-Colmenares et al (2021a) and Muñoz-Colmenares et al. (2021b).

Zooplankton assemblage and functional groups

In this study, a total of 169 species were taxonomically identified. Rotifers had the highest representation with 115 species, followed by cladocerans with 36 and copepods with 17. A complete list of zooplankton species present in the Ebro basin during this study and their functional traits can be found in Supplementary material 1. Zooplankton´s total abundance inside reservoirs varied from 6.76 to 2757 ind. L^{-1}, meanwhile biomass varied from 0.45 to 1971 µg DW L^{-1}. Based on the functional traits and following the previous methodology, zooplankton species were divided into five functional groups (Fig. 2). Group 1 consisted of three species of large suspending filter calanoid copepods with an average biomass of 11.3 µg DW L^{-1}. Group 2 included five species of raptorial omnivorous-carnivorous cyclopoids copepods (biomass average 3.85 µg DW L^{-1}), being *A. americanus* the most representative species with highest occurrences and abundances. Group 3 was composed of 11 species of cladocerans with different filter strategies and an average biomass of 2.6 µg DW L^{-1}. For this group *Daphnia cucullata* and *D. longispina* were the cladocerans with the highest representation along the basin. Group 4 included 16 species of microphagous filter rotifers and had an average biomass of 0.14 µg DW L^{-1}. Finally, group 5 was integrated by 17 species of raptorial rotifers with 0.18 µg DW L^{-1} of average biomass.

Comparing the ZFG to the different categories in the trophic state and the ecological potential, we can observe that abundance in general increases along the eutrophication (Fig. 3). This is more evident with calanoids, cladocerans and microphagous rotifers with less abundance in oligotrophic and good or higher scenarios, increasing it in eutrophic and moderate-poor categories. Cyclopoids had a considerable increase in hypereutrophic reservoirs but was not reflected in the "poor" level. In the case of raptorial rotifers, a difference was not visible among the trophic status and ecological potential levels.

Statistical interpretation

The first and second axis of PCA explained the 59.6% of variance in the Ebro´s reservoirs (33.3% and 26.4% respectively). The variables that had higher contribution of variation were phytoplankton abundance and reservoir volume. The PCA revealed that those reservoirs classified as oligotrophic had a similar position to those classified as good or higher (Fig. 4) and a similar case is found in those classified as eutrophic and moderate. Also, we can observe that a high number of mesotrophic reservoirs are shared between good or higher and moderate categories. For this reason, and in order to find the thresholds among these categories, mesotrophic reservoirs were removed from the "reduced dataset" and to compare if there are differences using the data of all reservoirs or "complete dataset".

The relationship between the physicochemical variables and the ZFG carried out through the RDA (Fig. 5), indicated that the two first axes explain the 17.75% of variance with a *p* value of 0.001 in the Monte Carlo permutation test. The first axis (13.02%), in its positive part was related to the variables that determine oligotrophic conditions (Secchi disk, reservoir volume and percentage of reservoir volume). To the contrary, on its negative part, variables were related to eutrophic conditions (TP and chlorophyll a). The second axis (4.73%) explained the relationship between environmental variables and functional groups. For this axis, the negative part was correlated to conductivity, ammonium and alkalinity, while cladocerans and calanoids were more related to these variables. Cyclopoids were related to temperature, chlorophyll a and TP, while microphagous rotifers were slightly related to these two last variables. Raptorial rotifers were related to water volume in the reservoirs. Pearson correlations indicated that most functional groups correlated significantly with variables that mainly determined both, trophic state and ecological potential of reservoirs (Table 2). In the case of chlorophyll a all groups were positively correlated, followed by total phosphorus and temperature with four groups each one. The variables that were negatively correlated were Secchi disk with three groups and the dissolved oxygen was only correlated with cyclopoids. This last group showed the strongest correlations to the environmental variables.

Random Forest and CARTs

The RF as screening tool, indicated that the functional group with the greatest importance to determine trophic status were cyclopoids and cladocerans. While for ecological potential were cyclopoids, calanoids and cladocerans (Fig.6). Then, we implemented RFs as a machine learning tool to predict the reservoirs' trophic status and ecological potential. Since the microcrustaceans had the highest success in determining trophic status and ecological potential, we used only their data separated by functional groups, and their combination as group and included rotifers to verify if there were differences (all functional groups). The predictions obtained from the models indicated that the accuracy was higher using the complete dataset for ecological potential and slightly better using calanoids and microcrustaceans data. Model´s accuracy was higher in the reduced dataset to determine trophic state with only cyclopoids followed by cladocerans and microcrustaceans data (Table 3).

We used a similar approach to set up the thresholds between categories comparing the information from the full and reduced datasets using CARTs. The results from the microcrustaceans were not very different among categories, full and reduced datasets, and functional groups, except calanoids were thresholds changed significantly between datasets in trophic status. In the case of rotifers, results were not very clear among groups and levels (Table 4). Since we obtained higher prediction accuracy in the full dataset, we used it to establish the reservoirs´ thresholds to separate good or higher from moderate categories in ecological potential: calanoids 1.4 ind. L^{-1}, cyclopoids 0.38 ind. L^{-1} and cladocerans 19.36 ind. L^{-1}. On the other hand, we used the reduced dataset to establish the thresholds to discern between oligotrophic and eutrophic conditions: calanoids 3.1 ind. L^{-1}, cyclopoids 0.76 ind. L^{-1} and cladocerans 19.04 ind. L^{-1}.

Discussion

There are several factors and variables that can affect and promote or decrease the zooplankton species and populations like nutrients, resources availability, quality food, fish predation, competition, and environmental variables (Carpenter et al. 2001, Lemmens et al. 2017, Ersoy et al. 2019). The environmental variables determine the water quality, while also, conditioning the species that may be present. Thus, the knowledge of which chemical and/or physical factors are related to groups has great importance.

Phytoplankton is the major zooplankton food source, and it can shape zooplankton communities' assembly, this is widely observed in many types of water bodies (Wetzel 2001, Vogt et al. 2013). According to our results, unsurprisingly chlorophyll a was the variable that had the highest correlation to the different functional groups. TP was correlated to most of ZFG in almost all trophic status and ecological potential categories, especially with cyclopoid copepods. TP is a limiting nutrient for phytoplankton and is the element to control and manage to prevent and reduce eutrophication in inland waters (Jeppensen et al. 2012). Also, it can affect species composition and has been positively correlated to biomass increases of zooplankton species in different latitudinal gradients in temperate (Gyllström et al. 2005), as well as subtropical and tropical zones (Wang et al. 2021). Temperature was the third variable most strongly associated with the functional groups, and this may affect zooplankton body size, abundance, biomass and composition, especially in a global warming scenario (Cremona et al. 2020). The conductivity, dissolved oxygen and Secchi disk visibility were related to some groups in our study and may greatly influence group composition (Sun et al. 2019).

A high TP concentration is strongly linked to the appearance of cyanobacterial blooms (Ger et al. 2014). These blooms can affect the zooplankton at different levels, e. g., by producing cyanotoxins (Nandini et al. 2020), or by their low nutritional quality as food or by being inedible on some occasions (De Bernardi et al. 1990, Engström et al. 2001). Krztoń and Kosiba (2020) show that cyanobacterial blooms negatively affect all densities of different ZFG exposed to short-lasting times in different waterbodies. Nevertheless, when ZFG were exposed in a long-lasting exposure, few groups increased their abundances. One of these groups were predatory organisms, mainly composed by cyclopoid copepods. Our results showed that in reservoirs with a higher degree of eutrophy and a high amount of TP this group increased considerably. This is in accordance with studies that showed them as an indicator of low water quality (Pinto-Coelho et al. 2005, Perbiche-Neves et al. 2021).

Therefore, the presence and high densities of this functional group can be a valuable component to detect eutrophicated environments and those that may be or were exposed to cyanobacterial blooms.

In the studies performed with ZFG, few have been focused on determining the trophic status and ecological potential. In the Cavado basin, located in Portugal, functional groups were analyzed in reservoirs along a wide trophic gradient (Almeida et al. 2020). It was found that ZFG were sensitive to changes. When the trophic status increased, the populations changed from large to small size organisms, and this could be a useful tool for the WFD (Almeida et al. 2020). We observed this pattern along the reservoirs in the Ebro watershed, but also, it has been noted in others water bodies with different characteristics and water quality. For example, the environments classified as oligotrophic, had a predominance of large filter-feeding cladocerans and filter-feeding calanoid copepods. In contrast, rotifers and small cladocerans were dominant in environments with high primary production microphagous (Goździejewska et al. 2021). Besides, with an approach of using the main zooplankton groups as water quality indicators, microcrustaceans were quite useful differentiating between water quality levels (Muñoz-Colmenares et al. 2021a). Within microcrustaceans, the cladocerans were the most sensitive group and were able to separate all trophic state categories and differentiate between categories in ecological potential. This high sensitivity may be because cladocerans are the most dependent group to primary productivity, as seen in a study of many template lakes (Vogt et al. 2013).

In the case of rotifers, total abundances in general have been used effectively as indicators of trophic status (May and O´Hare, 2005, Ejsmont-Karabin 2012). However, in our approach, microphagous had low densities in oligotrophic reservoirs and increased slightly for mesotrophic and eutrophic reservoirs. One reason for this might be that these rotifers cannot compete effectively against cladocerans (Obertegger et al. 2011) and this could be a special occurrence in oligotrophic scenarios, where larger cladocerans were present and competition pressure was higher. Then, when the trophic gradient increases and there is a shift in species from large to small size cladocerans and availability of resources increase, microphagous densities enhance. While raptorial rotifers do not suffer this pressure from cladocerans, since they are more selective and can consume smaller algae (Obertegger and Manca 2011). This could be one of the reasons that this group didn’t show major changes in their densities throughout the reservoirs. In light of the WFD's interests to have proper tools to differentiate ecological potentials among waterbodies, especially between those good or higher and the others, the results from the present research indicated that microcrustaceans were quite effective, while rotifers were less sensitive.

Normally, lakes and reservoirs exhibit their higher trophic state and phytoplankton peak during summer (Lampert and Sommer 1997). A point to take in account for this study, is that reservoirs were sampled only during summer season and may be debatable if the trait approach would be useful during the remaining seasons or could provide different information. However, along a latitudinal gradient and different eutrophication stages, the zooplankton community has been studied by using functional feeding groups during the spring and autumn seasons mainly (see Chengxue et al. 2019, Kuczyńska-Kippen et al. 2020). Thus, like in summer, water transparency was negatively correlated and together with dissolved oxygen and conductivity, it was one of the most strongly related parameters. Nevertheless, during spring and autumn, the taxonomic approach was more sensitive than the functional (Kuczyńska-Kippen et al. 2020). This supports the idea that using data from summer periods with a functional trait approach could be a valid way to determine the water quality effectively than in other seasons. Nowadays, this data collection has been possible in some cases, to monitoring programs covering different seasons of the year.

Machine learning approach

A strong point of the monitoring programs that have been implemented in recent years, such as the one carried out in this study, is the more frequent acquisition of robust datasets and information from large and wide zones like regions and basins (see Gyllström et al 2005, García-Chicote et al. 2019, Montagud et al. 2019, Duggan et al. 2020, Almeida et al. 2020, Mukhortova et al. 2021, among others). In handling these datasets, lots of interesting information can be obtained. For example, the use of those datasets through machine learning to predict changes in water quality, climate change, introduction of exotic species and species occurrences could be an interesting approach to implement for example, in basins and different types of waterbodies.

Recently, the use of different machine learning models has had an increase in the field of ecology. Nowadays, there are several machine learning models to select from and to take into account for diverse purposes. The RF method, moreover, its flexibility with the input data, makes it one of the best predictive models in assessing ecological quality in waters (Visser et al. 2022). Throughout aquatic ecology, RF has been used to determine which variables have a higher importance for several purposes, i.e., abiotic and biotic factors in projection for phytoplankton-cyanobacterial changes (Kakouei et al. 2021) and cyanobacterial blooms (Yu et al. 2022) or as a screening tool, using zooplankton data to include the most important groups in more complex models (Paquette et al. 2022). Here we used a similar approach, determining which ZFG had the highest ability to determine the trophic status and ecological potential and in terms of groups, microcrustaceans were more apt determiners than rotifers in general.

RFs can be used to perform predictions and build models with the original data. Since these models are predictions, they will always have an error, even more when they are built with data from natural systems. Nevertheless, the models with high predictive power can be useful. A large study done in the USA, predicting trophic status with chlorophyll a data had an accuracy range from 39%-82% (Hollister et al. 2016). Within this range similar was done to predict phytoplankton and phytobentos (35-81%) (Szomolányi and Clement, 2022) and up to 59% for zooplankton groups (Kakouei et al. 2022). In our different predictive models, the accuracy varied between 41% to 77%. This suggest that our models' results are congruent with previous studies in aquatic environments and could be used to predict ecological potential in reservoirs using zooplankton data.

Since the RFs are based on large numbers of CARTs that work individually to obtain a consensus, we used the results from the CARTs to establish the thresholds among categories. Therefore, we propose the use of calanoids, cyclopoids and cladocerans abundances obtained throughout CARTs as guide and a pathway to determine the ecological potential within the WFD based on the following. First, they were the groups or variables with higher importance in the random forest and they had the predictive models with better accuracy. Second, microcrustaceans compared to microphagous and raptorial rotifers are normally easier to identify at a group level. Thus, a deep knowledge is not required to discriminate among calanoids, cyclopoids and cladocerans, also features such as body size, shape and articulate appendices provide a good guide to differentiate them. Additionally, for rotifers, generally a deeper knowledge is needed in genera and/or trophi shape to define if a species belongs to microphagous or raptorial group.

Due to the fact that some reservoirs were not registered, the presence of copepods or cladocerans, which may be due to fish predation (Ersoy et al. 2019). Here, we recommend the use of not only one group to avoid false results, but all three (calanoids, cyclopoids and cladocerans) to determine the trophic status or ecological potential, Thus, the use of all available ZFG, will provide a better waterbody classification.

Hence, with the data obtained from the present research, joint with other studies we propose that zooplankton can be a useful water quality indicator. This found using the ZFG approach, or species presence (Montagud et al. 2019, García-Chicote et al. 2019, Muñoz-Colmenares et al. 2021b) or zooplankton abundances (García-Chicote et al. 2018, Stamou et al. 2021, Muñoz-Colmenares et al. 2021a,). In the case of the Ebro basin, low densities of large filter-feeding groups such as calanoid copepods and large cladocerans like *D. longispina* are indicators of good water quality (oligotrophy and good or higher ecological potential). In contrast, high abundances of small-sized species of cladocerans and raptorial copepods such as *A. americanus* are indicators of eutrophication and moderate-poor ecological potential. Nevertheless, their use and future research should be improved in different basins, regions, and climates to update the current models or create new ones to delimit the abundance thresholds among the different levels. Moreover, in an alternative scenario where those species are not present in the waterbody, then species that have a similar role inside the ecosystem and their functional group (large filters microcrustaceans and raptorial copepods) should be assessed and proved. Finally, we suggest that at least calanoids, cyclopoids, cladocerans and indicator species, as many authors mentioned in this article and others suggest, zooplankton should be included as BQEs inside the WFD monitoring programs.

Conclusions

The results from our study show that the data obtained from a functional groups-trait approach gives valuable information about the zooplankton community. Furthermore, this approach can be used as water quality indicator in reservoirs, specifically to distinguish among the different levels of trophic state and ecological potential. Moreover, ZFG in conjunction with the presence of indicator species and abundances, can provide high-quality information about the environment. ZFG could complement the information provided by phytoplankton and their indices as a tool for a better characterization and management of water bodies. The accuracy models based on RF had a 41% to 77% predictive power and were better to classify reservoirs using the complete dataset for trophic status and the reduced dataset for ecological potential. Here we established the thresholds to determine the ecological potential as an alternative way using ZFG and CARTs to classify reservoirs. The thresholds to determine good or higher status from moderate in ecological potential; calanoids <1.4 ind. L^{-1}, cyclopoids <0.38 ind. L^{-1} and cladocerans <19.36 ind. L^{-1}. The thresholds to discern between oligotrophic and eutrophic conditions were: calanoids <3.1 ind. L^{-1}, cyclopoids <0.76 ind. L^{-1} and cladocerans <19.04 ind. L^{-1}. Finally, we make a *call for action* and invite anyone who possesses any data related to zooplankton and wants to contribute and collaborate with us to improve the models and thresholds of trophic state and ecological potential in freshwater systems.

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Conflicts of Interest

No potential conflict of interest was reported by the authors. The funders had no role in the design of the study, in the collection, analyses, interpretation of data, in the writing of the manuscript, or in the decision to publish results.

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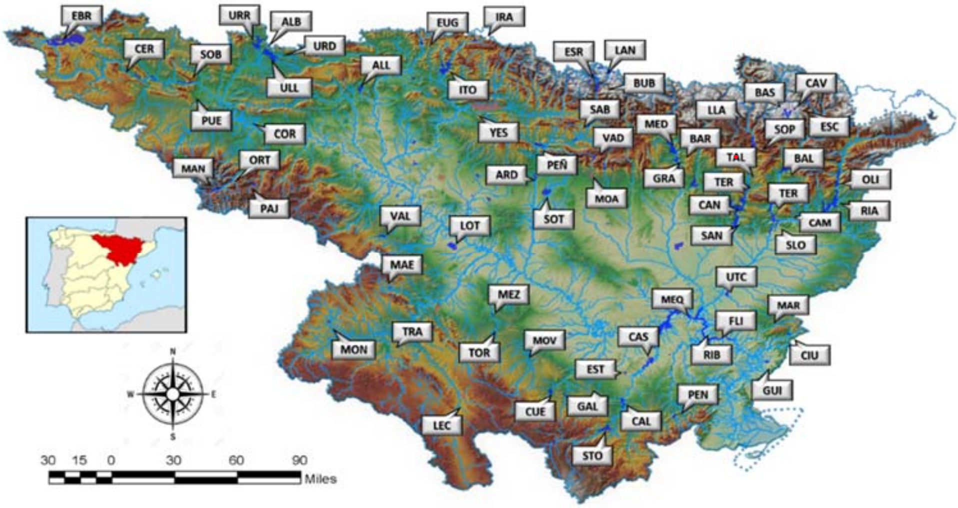


Figure 1. Map of Ebro watershed with approximate location of the studied reservoirs. Reservoirs´ abbreviations: ALB Albiña, ALL Alloz, ARD Ardisa, BAL Balaguer, BAR Barasona, BAS Baserca, BUB Búbal, CAL Calanda, CAM Camarasa, CAN Canelles, CAS Caspe, CAV Cavallers, CER Cereceda, CIU Çiurana, COR El Cortijo, CUE Foradada, EBR Ebro, ESC Escales, ESR Escarra, EST Alcañiz, EUG Eugui, FLI Flix, GAL Gallipuén, GRA El Grado, GUI Guiamets, IRA Irabia, ITO Itoiz, LAN Lanuza, LEC Lechago, LLA Llauset, LOT La Loteta, MAE Maidevera, MAN Mansilla, MAR Margalef, MED Mediano, MEQ Mequinenza, MEZ Mezalocha, MOA Montearagon, MON Vicarías, MOV Moneva, OLI Oliana, ORT Ortigosa, PAJ Pajares, PEÑ La Peña, PEN Pena, PUE Puentelarra, RIA Rialb, RIB Ribarroja, SAB Sabiñanigo, SAN Santa Ana, SLO San Lorenzo, SOB Sobrón, SOP Soperia, SOT Sotonera, STO Santolea, TAL Talrn, TER Terradets, TOR Las Torcas, TRA Tranquera, ULL Ullivari, URD Urdalur, URR Urrunaga, UTC Utexa seca, VAD Vadiello, VAL Val, YES Yesa. (Muñoz-Colmenares et al. (2021a), modified)

Figure 2. Dendrogram of zooplankton functional groups from the Ebro basin using only the dominant species. Every group is separated using different colors. Red = Calanoids copepods, Gold = Cyclopoids copepods, Purple = Cladocerans, Blue = Microphagous rotifers and Green = Raptorial rotifers.

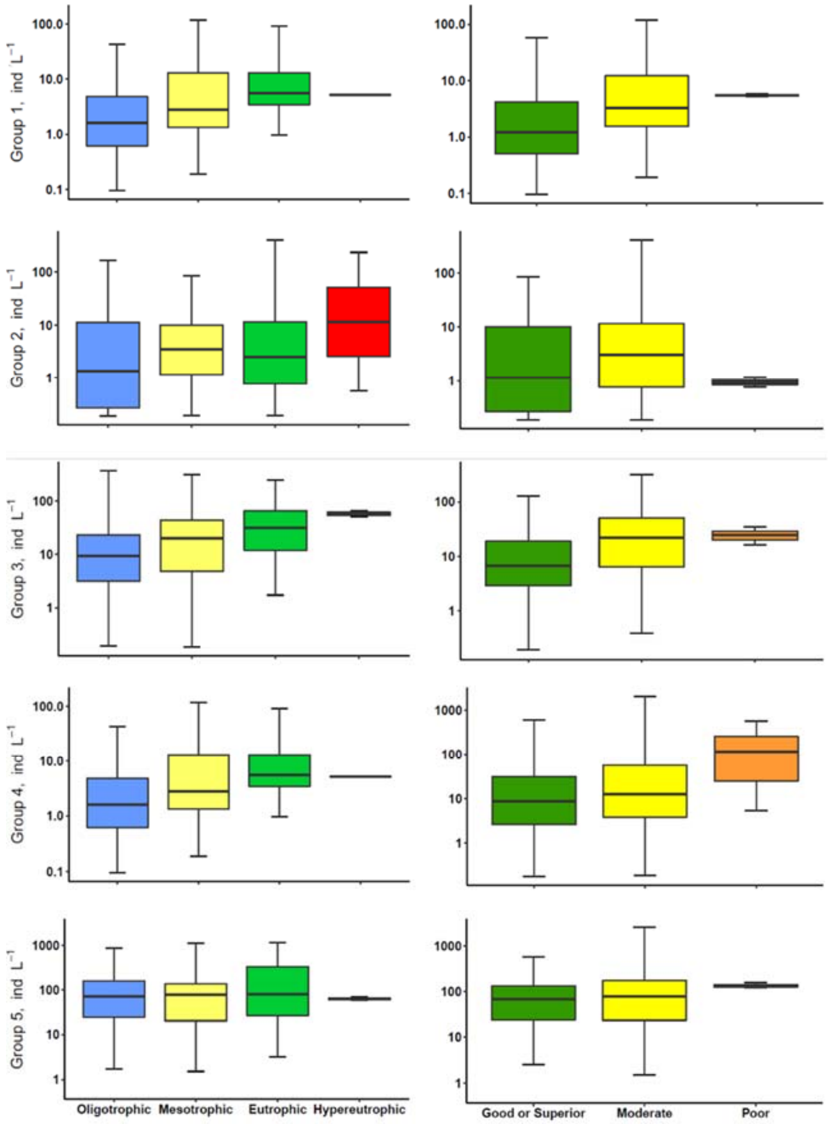


Figure 3. Boxplots of functional groups abundance, trophic status (**left side**), ecological potential (**right side**). The box binds the interquartile range (IQR, 25–75 percentile), the horizontal line inside the box indicates the median, and whiskers (error bars) indicate the 90th above and 10th below percentiles. Group 1 = Calanoids copepods, Group 2 = Cyclopoids copepods, Group 3 = Filter cladocerans, Group 4 = Microphagous rotifers, group 5 = Raptorial rotifers.

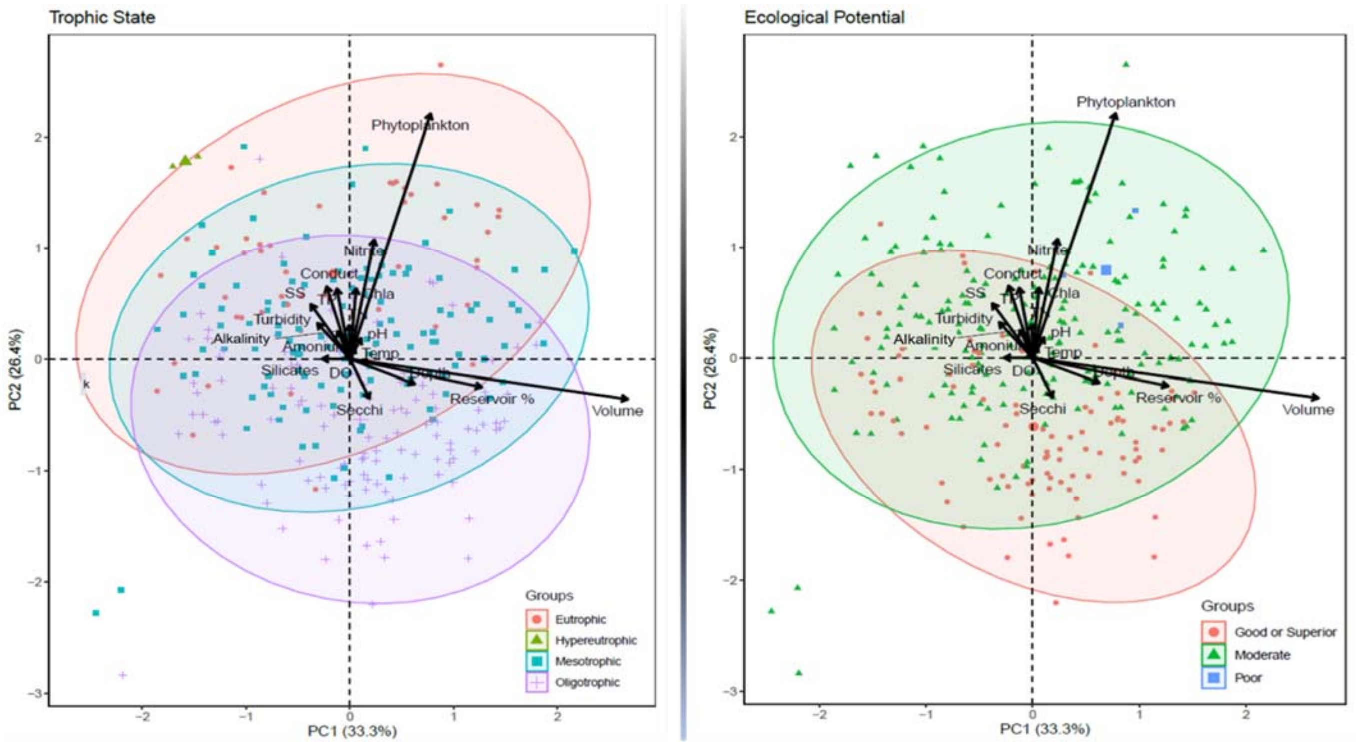


Figure 4. PCA of environmental variables in the Ebro´s reservoirs, left side: arranged based on their trophic status, right side: arranged based on their ecological potential. Samples are represented as dots, triangles, squares, or crosses. Chla = Chl-a, TP = Total phosphorus, Temp = temperature, DO = Dissolved oxygen, SS = suspended solids, Conduct = conductivity, Reservoir % = percentage of reservoir.

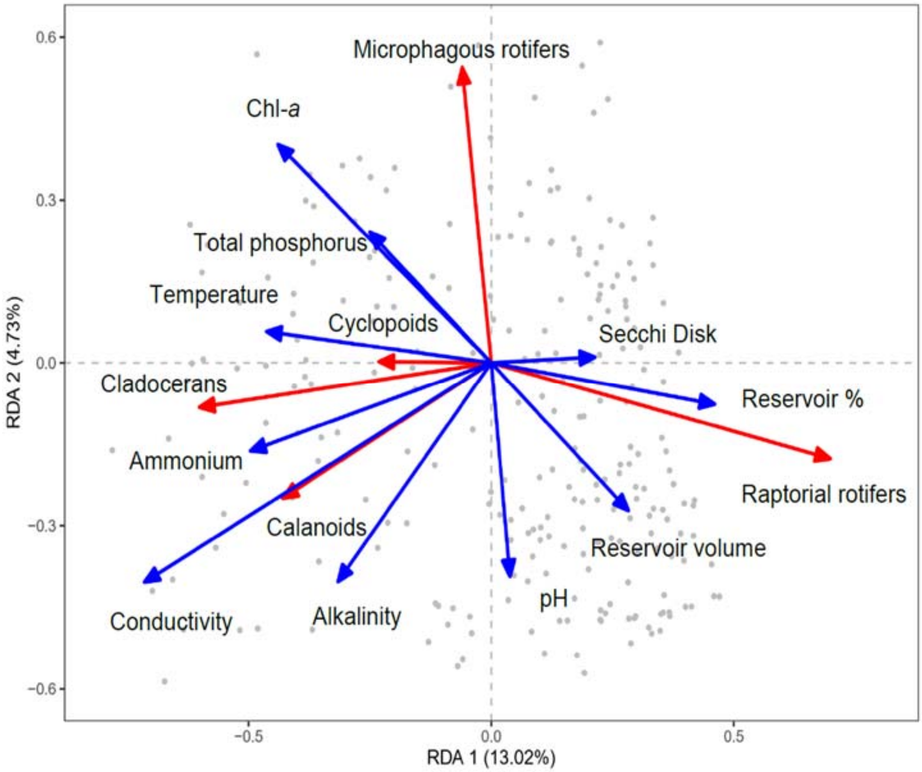
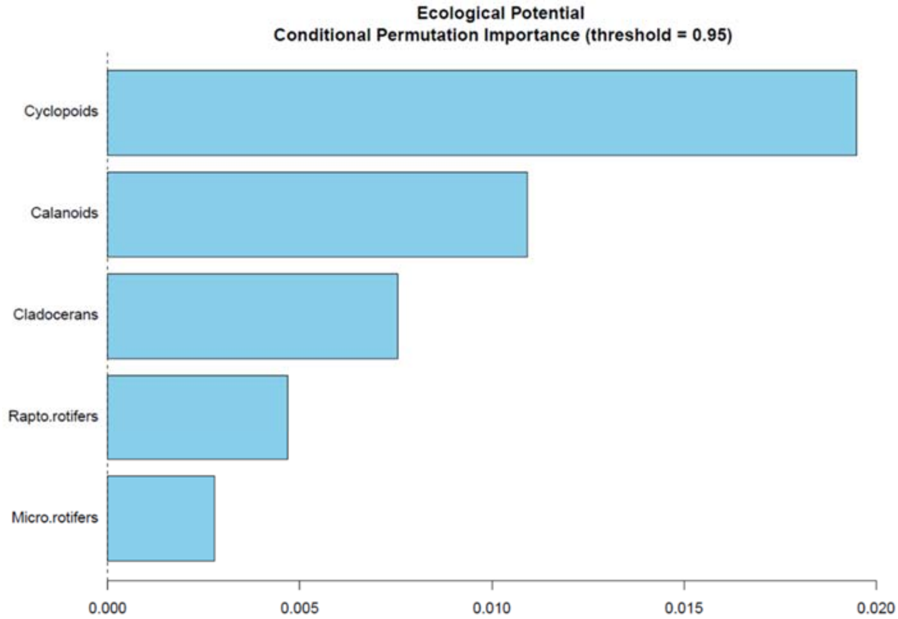


Figure 5. Redundancy Analysis (RDA) of environmental variables and zooplankton functional groups in Ebro´s reservoirs. Samples are represented as dots.



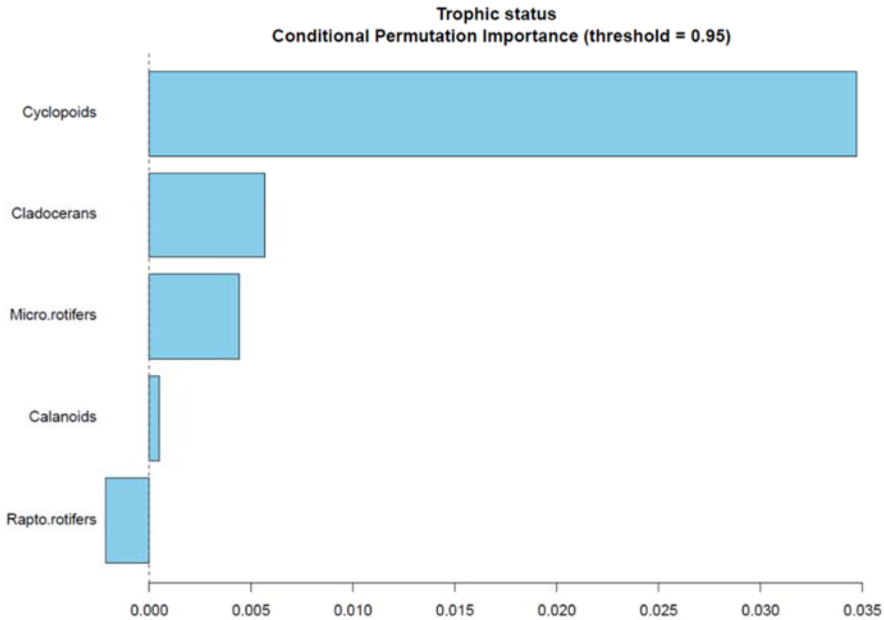


Figure 6. Conditional Permutation Importance of the trophic state and ecological potential through random forest. Rapto.rotifers = Raptorial rotifers, Micro.rotifers = Microphagous rotifers

Table 1. Zooplankton dominant species and their corresponding functional traits. Body weight in µg, Feeding type as filtration type: B—Bosminidae, D—Daphniidae, S—Sididae, and I—Ilyocriptidae

	(Trait #1)	(Trait #2)	(Trait #3)	(Trait #4)	(Trait #5)
Species	**Body weight**	**Habitat**	**Trophic group**	**Feeding type**	**Reproduction form**
Copepods					
Acanthocyclops americanus	5.15	Pelagic	Omnivore	Raptorial	Sexual
Cyclops sp.	5.20	Pelagic	Omnivore	Raptorial	Sexual
Cyclops vicinus	5.20	Pelagic	Omnivore	Raptorial	Sexual
Thermocyclops dybowskii	2.70	Pelagic	Omnivore	Raptorial	Sexual
Tropocyclops prasinus	1.00	Pelagic	Omnivore	Raptorial	Sexual
Copidodiaptomus numidicus	11.50	Pelagic	Herbivore	Suspension-feeders	Sexual
Neolovenula alluaudi	11.50	Pelagic	Herbivore	Suspension-feeders	Sexual
Eudiaptomus vulgaris	11.00	Pelagic	Herbivore	Suspension-feeders	Sexual
Cladocera					
Bosmina longirostris	1.30	Pelagic	Herbivore	B-Filtration	Asexual
Ceriodaphnia dubia	1.60	Pelagic	Herbivore	D-Filtration	Asexual
Ceriodaphnia pulchella	1.20	Pelagic	Herbivore	D-Filtration	Asexual
Daphnia cucullata	3.00	Pelagic	Herbivore	D-Filtration	Asexual
Daphnia galeata	3.40	Pelagic	Herbivore	D-Filtration	Asexual
Daphnia longispina	3.80	Pelagic	Herbivore	D-Filtration	Asexual
Daphnia parvula	3.00	Pelagic	Herbivore	D-Filtration	Asexual
Daphnia pulicaria	3.80	Pelagic	Herbivore	D-Filtration	Asexual
Diaphanosoma brachyurum	2.50	Pelagic	Herbivore	S-Filtration	Asexual
Diaphanosoma mongolianum	2.50	Pelagic	Herbivore	S-Filtration	Asexual
Diaphanosoma sp.	2.50	Pelagic	Herbivore	S-Filtration	Asexual
Rotifera					
Anuraeopsis fissa	0.05	Pelagic	Herbivore	Microphagous	Asexual
Ascomorpha ovalis	0.06	Pelagic	Herbivore	Raptorial	Asexual
Ascomorpha saltans	0.06	Pelagic	Herbivore	Raptorial	Asexual
Asplanchna girodi	0.70	Pelagic	Carnivorous	Raptorial	Asexual
Asplanchna priodonta	0.75	Pelagic	Omnivore	Raptorial	Asexual
Collotheca pelagica	0.03	Littoral	Herbivore	Microphagous	Asexual
Collotheca sp.	0.03	Pelagic	Herbivore	Microphagous	Asexual
Conochilus dossuarius	0.10	Pelagic	Herbivore	Microphagous	Asexual

Conochilus natans	0.10	Pelagic	Herbivore	Microphagous	Asexual
Conochilus sp.	0.10	Pelagic	Herbivore	Microphagous	Asexual
Conochilus unicornis	0.10	Pelagic	Herbivore	Microphagous	Asexual
Gastropus stylifer	0.20	Pelagic	Herbivore	Raptorial	Asexual
Hexarthra fennica	0.21	Pelagic	Herbivore	Microphagous	Asexual
Hexarthra intermedia	0.21	Pelagic	Herbivore	Microphagous	Asexual
Hexarthra mira	0.21	Pelagic	Herbivore	Microphagous	Asexual
Hexarthra oxyuris	0.21	Pelagic	Herbivore	Microphagous	Asexual
Kellicotia longispina	0.60	Pelagic	Herbivore	Microphagous	Asexual
Keratella cochlearis	0.05	Pelagic	Herbivore	Microphagous	Asexual
Keratella cochlearis tecta	0.05	Pelagic	Herbivore	Microphagous	Asexual
Keratella quadrata	0.12	Pelagic	Herbivore	Microphagous	Asexual
Polyarthra dolichoptera	0.05	Pelagic	Herbivore	Raptorial	Asexual
Polyarthra euryptera	0.13	Pelagic	Herbivore	Raptorial	Asexual
Polyarthra luminosa	0.05	Pelagic	Herbivore	Raptorial	Asexual
Polyarthra major	0.13	Pelagic	Herbivore	Raptorial	Asexual
Polyarthra vulgaris	0.05	Pelagic	Herbivore	Raptorial	Asexual
Pompholyx sulcata	0.06	Pelagic	Detrivore	Microphagous	Asexual
Synchaeta kitina	0.05	Pelagic	Herbivore	Raptorial	Asexual
Synchaeta longipes	0.33	Pelagic	Herbivore	Raptorial	Asexual
Synchaeta oblonga	0.05	Pelagic	Herbivore	Raptorial	Asexual
Synchaeta pectinata	0.33	Pelagic	Herbivore	Raptorial	Asexual
Synchaeta stylata	0.10	Pelagic	Herbivore	Raptorial	Asexual
Trichocerca pusilla	0.03	Pelagic	Herbivore	Raptorial	Asexual
Trichocerca similis	0.03	Pelagic	Herbivore	Raptorial	Asexual

Table 2. Pearson's correlations between environmental variables and functional groups. TP—total phosphorus, CHLA—chlorophyll *a*, Temp—temperature, DO—dissolved oxygen, SD—Secchi disk. Significance: **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

	Coefficients				
Group	TP	CHLA	Temp	DO	SD
			Abundance		
Calanoids	0.12*	0.17***	0.23***		
Cyclopoids	0.32***	0.29***	0.22***	-0.14*	-0.15**
Cladocerans	0.19***	0.40***	0.36***		-0.18**
Microphagous rotifers	0.16**	0.31***	0.17**		-0.12*
Raptorial rotifers		0.12**			

Table 3. Random Forest models and their accuracies. In bold are the highest accuracies for each category.

	Trophic state		Ecological Potential	
Full dataset	Accuracy %	Kappa	Accuracy %	Kappa
Calanoids	40.68%	0.09	**66.10**%	**0.08**
Cyclopoids	**54.25**%	**0.24**	59.32%	0.06
Cladocerans	45.76%	0.13	61.02%	0.12
Microcrustaceans	**51.01%**	**0.19**	**64.41%**	**0.13**
All functional groups	50.85%	0.18	62.71%	0.08
Reduced dataset				
Calanoids	71.43%	0.40	54.29%	0.13
Cyclopoids	**77.14**%	**0.47**	54.29%	0.08
Cladocerans	**74.29%**	**0.42**	45.71%	0.08
Microcrustaceans	**74.29%**	**0.42**	**57.14%**	**0.17**
All functional groups	68.57%	0.25	**65.71%**	**0.31**

Table 4. Classification regression trees and the abundance thresholds (ind. L^{-1}). In bold are the selected ZFG and thresholds to determine the trophic status and ecological potential.

	Trophic State				Ecological Potential			
	Full dataset		Reduced dataset		Full dataset		Reduced dataset	
	Oligotrophic	Mesotrophic	Oligotrophic	Eutrophic	Good or superior	Moderate	Good or superior	Moderate
Calanoids	< 27.90	> 27.90	**< 3.10**	**> 3.10**	**< 1.40**	**> 1.40**	< 2.10	> 2.10
Cyclopoids	< 0.76	> 0.76	**< 0.76**	**> 0.76**	**< 0.38**	**> 0.38**	< 0.19	> 0.19
Cladocerans	< 18.96	> 18.96	**< 19.04**	**> 19.04**	**< 19.36**	**> 19.36**	< 19.04	> 19.04
Microphaogus Rotifers	< 0.75	> 0.75	< 192	> 192	< 0.75	> 0.75	< 162	> 162
Raptorial Rotifers	< 931	> 931	< 914	> 914	< 583	> 583	< 620	> 620
All functional groups	< 757	> 757	< 714	> 714	< 714	> 714	< 260	> 260

Supplementary material from published papers

Supplementary Material from published papers

Chapter 2:

Can zooplankton species be used as indicators of trophic status and ecological potential of reservoirs?

Muñoz-Colmenares, M.E., Soria, J.M., and Vicente E. 2021.

Aquatic Ecology, 55, 1143–1156.

https://doi.org/10.1007/s10452-021-09897-8.

Supplementary table 1: Ebro Basin reservoirs trophic state and ecological potential. TS = Trophic State, EP = Ecological potential.

		2010		2011		2012		2013		2014	
Name	Code	TS	EP	TS	EP	TS	EP	TS	EP	TS	EP
Albiña	ALB					Oligotrophic	Moderate			Oligotrophic	Moderate
Alloz	ALL	Mesotrophic	Good or superior					Oligotrophic	Good or superior		
Ardisa	ARD	Eutrophic	Moderate			Eutrophic	Moderate				
Balaguer	BAL			Mesotrophic	Good or superior	Mesotrophic	Moderate			Mesotrophic	Good or superior
Barasona	BAR	Mesotrophic	Moderate	Oligotrophic	Good or superior	Oligotrophic	Good or superior			Oligotrophic	Good or superior
Baserca	BAS							Oligotrophic	Good or superior	Oligotrophic	Good or superior
Búbal	BUB							Oligotrophic	Good or superior		
Calanda	CAL	Oligotrophic	Moderate	Oligotrophic	Good or superior	Oligotrophic	Good or superior			Oligotrophic	Good or superior
Camarasa	CAM			Oligotrophic	Good or superior					Oligotrophic	Good or superior
Canelles	CAN							Oligotrophic	Good or superior		
Caspe	CAS	Mesotrophic	Moderate	Mesotrophic	Moderate	Oligotrophic	Moderate			Mesotrophic	Moderate
Cavallers	CAV										
Cereceda	CER			Eutrophic	Moderate	Eutrophic	Moderate				
Çiurana	CIU			Oligotrophic	Good or superior	Oligotrophic	Good or superior			Oligotrophic	Good or superior
El Cortijo	COR			Eutrophic	Moderate	Eutrophic	Moderate				
Foradada	CUE	Mesotrophic	Moderate	Mesotrophic	Moderate	Eutrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate
Ebro	EBR	Mesotrophic	Moderate	Eutrophic	Moderate	Mesotrophic	Moderate	Oligotrophic	Moderate	Mesotrophic	Moderate
Escales	ESC							Oligotrophic	Good or superior		
Escarra	ESR										
Alcañiz	EST										
Eugui	EUG			Oligotrophic	Good or superior			Oligotrophic	Good or superior		
Flix	FLI	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate			Mesotrophic	Moderate
Gallipuén	GAL	Mesotrophic	Moderate	Eutrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Good or superior	Oligotrophic	Good or superior
El Grado	GRA							Oligotrophic	Good or superior		
Guiamets	GUI	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate	Oligotrophic	Moderate

		2015		2016		2017		2018		2019	
Name	Code	TS	EP	TS	EP	TS	EP	TS	EP	TS	EP
Albiña	ALB	Mesotrophic	Moderate	Mesotrophic	Moderate					Oligotrophic	Moderate
Alloz	ALL	Oligotrophic	Moderate			Oligotrophic	Moderate				
Ardisa	ARD										
Balaguer	BAL	Mesotrophic	Good or superior								
Barasona	BAR			Mesotrophic	Moderate	Oligotrophic	Moderate	Oligotrophic	Good or superior		
Baserca	BAS										
Búbal	BUB	Oligotrophic	Good or superior								
Calanda	CAL			Oligotrophic	Moderate	Oligotrophic	Moderate				
Camarasa	CAM					Oligotrophic	Good or superior				
Canelles	CAN			Oligotrophic	Good or superior						
Caspe	CAS	Oligotrophic	Moderate	Mesotrophic	Moderate						
Cavallers	CAV	Oligotrophic	Good or superior	Oligotrophic	Good or superior						
Cereceda	CER										
Çiurana	CIU					Oligotrophic	Good or superior				
El Cortijo	COR										
Foradada	CUE	Oligotrophic	Moderate			Mesotrophic	Moderate	Eutrophic	Moderate	Eutrophic	Moderate
Ebro	EBR	Mesotrophic	Moderate	Oligotrophic	Moderate	Mesotrophic	Moderate	Oligotrophic	Moderate	Oligotrophic	Moderate
Escales	ESC			Oligotrophic	Good or superior						
Escarra	ESR	Oligotrophic	Good or superior								
Alcañiz	EST	Mesotrophic	Good or superior			Mesotrophic	Moderate	Mesotrophic	Moderate	Oligotrophic	Good or superior
Eugui	EUG					Oligotrophic	Moderate				
Flix	FLI	Mesotrophic	Moderate			Mesotrophic	Moderate	Eutrophic	Moderate		
Gallipuén	GAL					Mesotrophic	Moderate			Mesotrophic	Moderate
El Grado	GRA			Oligotrophic	Good or superior						
Guiamets	GUI	Mesotrophic	Poor	Mesotrophic	Moderate						

		2010		2011		2012		2013		2014	
Name	Code	TS	EP	TS	EP	TS	EP	TS	EP	TS	EP
Irabia	IRA					Oligotrophic	Good or superior			Oligotrophic	Moderate
Itoiz	ITO			Oligotrophic	Good or superior			Oligotrophic	Good or superior		
Lanuza	LAN							Oligotrophic	Good or superior		
Lechago	LEC	Oligotrophic	Moderate	Mesotrophic	Moderate	Eutrophic	Moderate	Oligotrophic	Good or superior		
Llauset	LLA										
La Loteta	LOT			Oligotrophic	Good or superior	Eutrophic	Moderate	Mesotrophic	Moderate	Eutrophic	Moderate
Maidevera	MAE									Mesotrophic	Moderate
Mansilla	MAN	Oligotrophic	Good or superior					Oligotrophic	Good or superior		
Margalef	MAR					Mesotrophic	Moderate	Eutrophic	Moderate	Mesotrophic	Moderate
Mediano	MED							Oligotrophic	Good or superior		
Mequinenza	MEQ	Oligotrophic	Moderate	Oligotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate
Mezalocha	MEZ			Eutrophic	Moderate	Hypereutrophic	Moderate	Mesotrophic	Good or superior	Mesotrophic	Good or superior
Montearagon	MOA					Oligotrophic	Moderate			Oligotrophic	Good or superior
Vicarías	MON			Oligotrophic	Good or superior	Mesotrophic	Moderate			Mesotrophic	Moderate
Moneva	MOV					Eutrophic	Moderate	Oligotrophic	Good or superior		
Oliana	OLI	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate			Eutrophic	Moderate
Ortigosa	ORT	Oligotrophic	Good or superior					Oligotrophic	Good or superior		
Pajares	PAJ	Oligotrophic	Good or superior	Oligotrophic	Good or superior					Oligotrophic	Good or superior
La Peña	PEÑ	Eutrophic	Moderate	Eutrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate
Pena	PEN	Oligotrophic	Good or superior					Oligotrophic	Good or superior	Oligotrophic	Good or superior
Puentelarra	PUE					Mesotrophic	Moderate				
Rialb	RIA	Mesotrophic	Moderate	Oligotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate
Ribarroja	RIB	Eutrophic	Moderate	Eutrophic	Moderate	Eutrophic	Moderate			Eutrophic	Moderate
Sabiñanigo	SAB							Oligotrophic	Good or superior		
Santa Ana	SAN	Mesotrophic	Good or superior	Oligotrophic	Good or superior					Oligotrophic	Good or superior

Name	Code	2015 TS	2015 EP	2016 TS	2016 EP	2017 TS	2017 EP	2018 TS	2018 EP	2019 TS	2019 EP
Irabia	IRA	Oligotrophic	Moderate			Oligotrophic	Moderate				
Itoiz	ITO					Oligotrophic	Good or superior				
Lanuza	LAN	Oligotrophic	Good or superior								
Lechago	LEC	Mesotrophic	Moderate			Mesotrophic	Moderate	Oligotrophic	Moderate		
Llauset	LLA	Oligotrophic	Good or superior			Oligotrophic	Good or superior				
La Loteta	LOT									Mesotrophic	Moderate
Maidevera	MAE	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate				
Mansilla	MAN			Oligotrophic	Moderate	Oligotrophic	Moderate				
Margalef	MAR	Oligotrophic	Moderate	Oligotrophic	Moderate						
Mediano	MED			Oligotrophic	Good or superior						
Mequinenza	MEQ	Oligotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate				
Mezalocha	MEZ	Mesotrophic	Good or superior	Eutrophic	Moderate	Eutrophic	Moderate	Oligotrophic	Moderate		
Montearagon	MOA										
Vicarías	MON	Mesotrophic	Moderate			Oligotrophic	Moderate	Mesotrophic	Moderate		
Moneva	MOV			Mesotrophic	Moderate	Hypereutrophic	Moderate			Eutrophic	Moderate
Oliana	OLI	Mesotrophic	Moderate	Eutrophic	Poor	Eutrophic	Moderate	Mesotrophic	Moderate	Oligotrophic	Moderate
Ortigosa	ORT			Oligotrophic	Good or superior	Oligotrophic	Good or superior				
Pajares	PAJ			Oligotrophic	Good or superior						
La Peña	PEÑ	Eutrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate				
Pena	PEN			Oligotrophic	Good or superior						
Puentelarra	PUE										
Rialb	RIA	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate
Ribarroja	RIB	Mesotrophic	Moderate			Eutrophic	Moderate	Eutrophic	Moderate	Eutrophic	Moderate
Sabiñanigo	SAB										
Santa Ana	SAN			Oligotrophic	Good or superior						

		2010		2011		2012		2013		2014	
Name	Code	TS	EP	TS	EP	TS	EP	TS	EP	TS	EP
San Lorenzo	SLO	Mesotrophic	Good or superior							Mesotrophic	Good or superior
Sobrón	SOB	Eutrophic	Moderate	Eutrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate
Soperia	SOP										
Sotonera	SOT	Mesotrophic	Good or superior	Mesotrophic	Moderate			Oligotrophic	Good or superior		
Santolea	STO	Oligotrophic	Good or superior					Oligotrophic	Good or superior		
Talrn	TAL							Mesotrophic	Good or superior		
Terradets	TER	Mesotrophic	Moderate			Mesotrophic	Moderate			Eutrophic	Moderate
Las Torcas	TOR	Mesotrophic	Good or superior	Mesotrophic	Good or superior			Oligotrophic	Good or superior		
Tranquera	TRA	Mesotrophic	Moderate			Eutrophic	Moderate	Mesotrophic	Moderate	Oligotrophic	Moderate
Ullivari	ULL	Oligotrophic	Good or superior			Mesotrophic	Moderate	Oligotrophic	Good or superior		
Urdalur	URD										
Urrunaga	URR	Mesotrophic	Moderate	Oligotrophic	Moderate	Mesotrophic	Moderate	Oligotrophic	Good or superior		
Utexa seca	UTC			Eutrophic	Moderate	Eutrophic	Moderate				
Vadiello	VAD	Mesotrophic	Good or superior							Oligotrophic	Good or superior
Val	VAL			Eutrophic	Moderate	Eutrophic	Moderate	Eutrophic	Moderate	Eutrophic	Moderate
Yesa	YES	Oligotrophic	Good or superior							Oligotrophic	Moderate

		2015		2016		2017		2018		2019	
Name	Code	TS	EP	TS	EP	TS	EP	TS	EP	TS	EP
San Lorenzo	SLO	Mesotrophic	Good or superior								
Sobrón	SOB	Mesotrophic	Moderate	Eutrophic	Moderate	Eutrophic	Moderate	Mesotrophic	Moderate	Eutrophic	Moderate
Soperia	SOP	Oligotrophic	Good or superior								
Sotonera	SOT	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate
Santolea	STO			Oligotrophic	Moderate						
Talrn	TAL			Oligotrophic	Good or superior						
Terradets	TER	Mesotrophic	Moderate			Mesotrophic	Moderate	Eutrophic	Moderate		
Las Torcas	TOR			Oligotrophic	Moderate	Oligotrophic	Good or superior				
Tranquera	TRA	Mesotrophic	Good or superior	Mesotrophic	Moderate	Mesotrophic	Moderate	Mesotrophic	Moderate	Eutrophic	Moderate
Ullivari	ULL			Oligotrophic	Moderate						
Urdalur	URD	Oligotrophic	Good or superior								
Urrunaga	URR			Mesotrophic	Moderate	Mesotrophic	Poor	Oligotrophic	Moderate		
Utexa seca	UTC			Hypereutrophic	Moderate					Eutrophic	Moderate
Vadiello	VAD					Oligotrophic	Good or superior				
Val	VAL	Eutrophic	Moderate	Eutrophic	Moderate	Eutrophic	Moderate	Eutrophic	Moderate	Eutrophic	Moderate
Yesa	YES	Oligotrophic	Good or superior			Oligotrophic	Moderate				

Supplementary table 2. List of zooplankton species in the Ebro Basin.

Rotifera	*Proales sp*
Class Bdelloidea	*Ptygura sp*
Bdelloids	*Squatinella rostrum*
Class Monogononta	*Synchaeta grandis*
Anuraeopsis fissa	*S. kitina*
Ascomorpha ecaudis	*S. longipes*
A. ovalis	*S. oblonga*
A. saltans	*S. pectinata*
Ascomorpha sp.	*S. stylata*
Asplanchna girodi	*S. tremula*
A. priodonta	*Synchaeta sp*
A. sieboldi	*Testudinella incisa*
Asplanchna sp	*T. mucronata*
Brachionus angularis	*T. patina*
B. bidentata	*Trichocerca cylindrica*
B. calyciflorus	*T. gracilis*
B. dimidiatus	*T. inermis*
B. havanaensis	*T. insignis*
B. plicatilis	*T. pusilla*
B. quadridentatus	*T. similis*
B. urceolaris	*T. tenuinor*
Cephalodella gibba	*T. tigris*
C. stenroosi	*Trichocerca sp*
Cephalodella sp	*Trichotria pocillum*
Collotheca pelagica	*T. tetractis*
Collotheca sp.	*Tripleuchlanis plicata*
Colurella colurus	
C. obtusa	**Crustacea**
C. uncinata	**Suborder Cladocera**
Conochilus dossuarius	*Alona affinis*

C. natans
C. unicornis
Conochilus sp
Dicranophorus sp
Encentrum sp
Eosphora sp
Euchlanis dilatata
Filinia longiseta
F. terminalis
Gastropus stylifer
Hexarthra fennica
H. intermedia
H. mira
H. oxyuris
Hexarthra sp.
Kellicottia longispina
Keratella cochlearis
K. cochlearis tecta
K. hiemalis
K. quadrata
K. tropica
Lecane aculeata
L. bulla
L. clara
L. closterocerca
L. cornuta
L. flexilis
L. furcata
L. inermis
L. luna
L. lunaris
A. guttata
A. quadrangularis
A. rectangula
Alona sp.
Alonella exigua
A. nana
Bosmina longirostris
Ceriodaphnia dubia
C. laticaudata
C. pulchella
C. quadrangula
Chydorus sphaericus
Daphnia cucullata
D. curvirostris
D. galeata
D. longispina
D. magna
D. parvula
D. pulicaria
Daphnia rosea
Diaphanosoma brachyurum
D. lacustris
D. mongolianum
Diaphanosoma sp.
Holopedium gibberum
Ilyocryptus sordidus
Leydigia acanthocercoides
L. leydigi
L. quadrangularis
Macrothrix hirsuticornis
M. laticornis

L. puriformis	*Moina micrura*
L. stenroosi	*Oxyurella tenuicaudis*
L. stichaea	*Phrixura leei*
L. tenuiseta	*Sida crystalina*
Lecane sp	
Lepadella acuminata	**Copepoda**
L. ovalis	**Order Cyclopoida**
L. patella	*Acanthocyclops americanus*
L. rhomboides	*A. robustus*
Lophocaris salpina	*Cyclops abyssorum*
L. oxysternon	*C. lacustris*
Macrochaetus subquadratus	*C. vicinus*
Monommata appendiculata	*Cyclops sp.*
Mytilina mucronata	*Eucyclops serrulatus*
Notholca acuminata	*Eucyclops sp*
N. squamula	*Macrocyclops albidus*
Notommata allantois	*Thermocyclops dybowskii*
N. copeus	*Tropocyclops prasinus*
Ploesoma hudsoni	**Order Calanoida**
P. lenticulare	*Copidodiaptomus numidicus*
P. truncatum	*Eudiaptomus vulgaris*
Polyarthra dolichoptera	*Neolovenula alluaudi*
P. euryptera	**Order Harpacticoida**
P. longiremis	Harpacticoids
P. luminosa	**Order Poecilostomatoida**
P. major	*Ergasilus sieboldi*
P. minor	*Neoergasilus japonicus*
Polyarthra vulgaris	
Polyarthra sp	**Mollusca**
Pompholyx sulcata	**Class Bivalvia**
P. triloba	*Dreissena polymorpha*

Supplementary Material

Chapter 3

The Use of Zooplankton Metrics to Determine the Trophic Status and Ecological Potential: An Approach in a Large Mediterranean Watershed.

Muñoz-Colmenares, M.E.; Sendra, M.D.; Sòria-Perpinyà, X.; Soria, J.M.; Vicente, E. 2021.

Water, 13, 2382.

https://doi.org/10.3390/w13172382

Supplementary Literature Table S1.

SP1. Average physical and chemical data from reservoirs of the Ebro Watershed. Samples= Number of years that a reservoir was sampled, Depth = reservoir maximum sampling depth, Volume = reservoir maximum volume, T = Temperature, DO = Dissolved oxygen, SD = Secchi disk depth, SS= Suspend solids, Chl-*a* = chlorophyll *a*, TP = Total phosphorus.

Code	Reservoir	Samples	Depth	Volume	T	DO	Conductivity	pH	SD	SS	Turbidity	Chl-*a*	PT
			(m)	(hm^3)	°C	(mg/L)	(µS/cm)		(m)	(mg/L)	(NTU)	(µg/L)	(µg/L)
ALB	Albiña	5	18.98	3.47	19.50	7.44	94	7.72	2.97	2.37	4.99	2.85	8.27
ALL	Alloz	4	38.13	51.64	21.53	8.61	640	8.21	3.05	3.11	2.41	1.68	5.63
ARD	Ardisa	2	7.30	1.41	18.75	9.30	277	8.43	0.55	25.52	15.92	1.09	33.45
BAL	Balaguer	4	4.25	0.95	19.81	9.02	242	8.05	2.41	6.37	3.77	2.99	13.82
BAR	Barasona	7	24.93	63.50	23.01	8.12	277	8.26	3.50	3.10	3.34	2.58	10.27
BAS	Baserca	2	31.25	15.00	13.31	9.24	38	7.58	11.70	0.50	0.99	1.32	1.54
BUB	Búbal	2	35.00	39.75	19.40	8.61	141	8.54	6.40	0.84	1.08	2.07	7.15
CAL	Calanda	6	26.20	26.96	23.15	7.63	599	8.22	3.78	2.93	2.07	1.32	6.70
CAM	Camarasa	3	58.47	116.84	19.65	8.83	186	8.37	5.10	1.86	3.12	1.76	6.66
CAN	Canelles	2	107.75	443.55	24.33	9.95	233	8.12	3.75	1.89	1.82	2.34	6.33
CAS	Caspe	6	24.08	39.52	23.76	7.29	1121	8.25	3.36	3.69	2.81	3.70	11.27
CAV	Cavallers	2	69.35	15.35	14.92	9.26	13	6.94	17.60	0.24	0.49	0.82	1.89
CER	Cereceda	2	11.15	1.62	19.40	8.65	241	8.14	1.23	10.74	7.82	7.01	33.18
CIU	Çiurana	4	36.45	9.50	19.38	9.66	536	8.27	6.08	1.95	1.90	1.23	6.80
COR	El Cortijo	2	7.70	0.90	21.35	8.10	467	8.01	1.25	10.16	7.06	2.17	95.86
CUE	Foradada	9	16.77	12.25	21.32	6.94	895	7.99	2.56	5.83	4.00	5.84	20.97
EBR	Ebro	10	17.74	392.24	20.38	7.44	205	8.08	3.38	2.53	3.36	4.35	15.93
ESC	Escales	2	90.00	142.30	20.92	9.94	175	8.62	6.20	1.29	1.49	2.53	4.83
ESR	Escarra	1	17.00	3.50	18.40	8.34	144	8.43	5.70	1.57	1.88	1.14	2.25
EST	Alcañiz	4	4.23	4.40	26.02	8.55	629	8.25	2.03	6.17	4.06	3.13	11.62

EUG	Eugui	3	30.33	16.02	20.79	7.81	164	8.02	5.03	1.36	2.33	2.12	10.37
FLI	Flix	7	8.43	3.73	22.58	4.59	978	7.92	4.16	2.14	2.83	1.53	60.86
GAL	Gallipuén	7	13.71	2.59	21.51	8.31	549	8.14	2.44	6.39	4.64	3.43	16.15
GRA	El Grado	2	68.10	314.05	19.90	8.50	236	8.27	5.75	1.41	1.76	1.01	2.32
GUI	Guiamets	7	31.99	7.13	23.12	9.49	405	8.34	2.48	4.70	4.59	4.68	14.21
IRA	Irabia	4	27.60	7.76	18.00	7.03	153	7.91	5.13	1.68	1.59	2.53	6.39
ITO	Itoiz	3	68.70	281.27	21.75	8.26	198	8.43	4.83	1.77	2.29	1.47	5.70
LAN	Lanuza	2	43.25	15.15	16.00	9.10	111	8.06	5.98	1.00	0.97	1.26	8.28
LEC	Lechago	7	10.26	2.73	19.76	6.38	1364	7.86	3.71	3.37	4.17	4.87	15.19
LLA	Llauset	2	64.40	15.20	11.27	9.16	55	7.75	16.00	0.36	0.49	0.61	1.63
LOT	La Loteta	5	10.98	42.86	21.51	8.15	950	8.22	1.44	11.07	8.68	4.32	21.31
MAE	Maidevera	4	33.38	14.94	21.93	8.89	403	8.22	2.40	5.54	3.94	5.87	13.96
MAN	Mansilla	4	35.08	37.17	20.18	7.89	151	8.23	4.55	1.56	1.52	2.33	9.85
MAR	Margalef	5	23.90	2.26	21.77	8.76	360	8.14	3.59	2.72	2.52	4.84	15.54
MED	Mediano	2	43.70	258.60	22.52	8.40	224	8.21	3.00	2.53	2.13	1.84	2.57
MEQ	Mequinenza	8	52.91	1151.85	24.94	5.99	1022	8.09	4.34	2.05	2.17	3.91	12.80
MEZ	Mezalocha	8	9.95	2.19	18.96	8.45	523	8.26	1.70	27.55	21.77	6.07	41.77
MOA	Montearagon	2	29.60	10.00	21.30	6.20	341	8.17	3.33	3.01	3.31	2.43	2.80
MON	Vicarías	6	6.85	4.55	22.66	7.00	844	8.02	1.71	7.93	7.94	1.91	9.28
MOV	Moneva	5	5.28	1.77	22.70	7.27	950	8.12	1.58	13.70	14.66	6.66	41.78
OLI	Oliana	9	47.15	68.13	22.15	7.76	214	8.48	3.12	3.78	3.59	8.79	22.08
ORT	Ortigosa	5	29.60	21.89	19.10	8.01	150	8.07	5.54	2.17	1.83	1.86	8.64
PAJ	Pajares	4	45.25	54.75	16.94	8.03	70	7.56	5.25	1.20	2.15	2.32	5.83
PEN	La Peña	4	25.65	13.30	18.52	9.29	322	8.34	4.13	2.20	1.63	1.55	3.85
PEÑ	Pena	8	11.14	11.17	20.58	8.14	279	8.18	1.09	9.41	10.00	3.88	20.90
PUE	Puentelarra	1	5.00	0.90	23.70	7.60	294	8.31	2.55	1.66	3.86	2.03	16.80
RIA	Rialb	10	56.83	315.19	23.13	7.68	218	8.33	2.56	2.96	3.43	5.30	15.64
RIB	Ribarroja	8	28.38	203.27	24.05	7.36	906	8.14	2.94	3.11	3.15	12.94	42.80
SAB	Sabiñanigo	1	10.80	0.90	14.60	9.09	204	7.83	2.60	22.62	1.97	0.01	7.24
SAN	Santa Ana	4	48.20	127.13	15.05	9.88	281	8.16	3.72	1.35	1.51	1.63	10.54

SLO	San Lorenzo	3	9.00	8.60	20.73	8.67	197	8.18	2.03	3.84	3.19	2.68	13.13
SOB	Sobrón	10	26.84	17.88	22.24	7.35	312	8.07	2.57	2.88	3.91	7.08	23.98
SOP	Sopeira	1	15.10	1.00	11.63	9.10	171	7.87	4.25	0.91	1.40	0.40	3.59
SOT	Sotonera	8	17.73	131.49	23.61	8.00	333	8.26	2.20	4.11	3.06	3.20	11.28
STO	Santolea	3	23.83	25.20	23.60	8.02	519	8.17	4.92	1.94	1.30	1.18	6.30
TAL	Talarn	2	47.65	165.10	19.65	9.19	173	8.55	4.46	2.09	1.69	3.60	8.49
TER	Terradets	6	12.33	28.16	18.00	8.36	173	8.14	0.93	20.79	19.29	1.83	25.96
TOR	Las Torcas	5	19.26	4.54	20.31	9.46	491	8.16	3.86	2.77	2.55	2.27	10.07
TRA	Tranquera	9	26.77	44.58	23.44	9.41	644	8.20	3.15	3.80	2.15	10.00	16.75
ULL	Ullivari	5	22.76	114.36	20.44	7.72	257	8.31	5.31	1.35	3.69	2.70	12.64
URD	Urdalur	1	36.00	5.46	14.80	8.75	119	7.76	5.80	1.17	3.46	1.59	3.81
URR	Urrunaga	7	17.06	56.18	20.78	7.41	180	8.22	4.36	1.36	3.05	3.33	16.65
UTC	Utexa seca	4	4.60	2.48	22.28	8.78	524	8.12	0.72	21.33	11.19	29.16	103.46
VAD	Vadiello	3	52.93	14.22	19.23	9.16	301	8.15	5.30	2.15	1.42	2.02	7.87
VAL	Val	9	40.99	17.81	22.12	10.20	400	8.38	1.71	8.27	5.80	29.74	39.94
YES	Yesa	4	44.60	359.64	21.94	8.58	250	8.21	2.91	2.14	2.01	1.68	4.97

Dissemination of thesis results at international symposiums, congresses or meetings

Oral presentations

Muñoz-Colmenares, M.E., Soria, J.M., and Vicente E. **2022**. Rotifers functional diversity in a large fluvial Mediterranean watershed. XVI International Rotifer Symposium. Zagreb, Croatia. 5-9 September.

Muñoz-Colmenares, M.E., Soria, J.M., and Vicente E. **2022**. Zooplankton functional groups as water quality indicators: application to reservoirs. 36th Congress of the International Society of Limnology (SIL). Berlin, Germany. 7-10 August.

Muñoz-Colmenares, M.E., Soria, J.M., and Vicente E. **2021**. Use of zooplankton metrics to determine the trophic status and ecological potential of reservoirs, an approach in a large Mediterranean river watershed. 35th Congress of the International Society of Limnology (SIL). Seoul, South Korea. 23-26 August. Online congress.

Muñoz-Colmenares M.E., Soria, X., Alfonso, T., Sendra, M.D., Soria, J.M. and Vicente E. **2020.** Zooplankton as an indicator of ecological potential: An experimental approach in the reservoirs of the Hydrographic Basin of the Ebro River. XX Congress of the Iberian Association of Limnology (AIL-2020) & III Ibero-American Congress of Limnology (CIL-2020). October 26-29. Murcia, Spain

Muñoz-Colmenares M.E., Vicente, E., Sendra, M.D., Soria, X. and Soria, J.M. **2019.** Monitoring of the trophic state of the Ebro reservoirs, 9 years of studies. Brazilian Congress of Limnology &

2nd Iberoamerican Congress of Limnology. August 4-9. Florianópolis, Santa Catarina, Brazil.

Muñoz-Colmenares M.E., Vicente, E., Sendra, M.D., Soria, X. and Soria, J.M. **2019.** Potentially toxic cyanobacteria in the Ebro Basin, a case study. 2nd Iberoamerican Congress and 6th Iberian Congress of Cyanotoxins. July 3-5. Murcia, Spain

Courses completed

2020. Theorical-Practique Course. “Introducción a la teledetección y aplicaciones medioambientales del programa europeo Copernicus. Calidad de aguas continentales”. February 3-4. Faculdade de Ciencias, Universidade de Porto. Portugal.

2020. International course and workshop: Neotropical zooplankton. November 9-30. Universidad del Cauca. Colombia. Held online.

Congresses and courses organizer

2021. III International Congress on Rivers and Wetlands (CRYH). Barranquilla, Colombia, October 25-29. Participation as “Logistic support and organizer”. University of the Coast. Online congress.

2019. Speaker and organizer of the Course “Limnology and Aquatic Ecology” (“Limnología y Ecología Acuática”). Held in the National University of the Altiplano-Puno. Biological Sciences Faculty. Puno, Peru.

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