METACOMMUNITIES AND BIODIVERSITY PATTERNS IN MEDITERRANEAN TEMPORARY PONDS: THE ROLE OF POND SIZE, NETWORK CONNECTIVITY AND DISPERSAL MODE

Irene Tornero Pinilla

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Universitat de Girona

DOCTORAL THESIS

Metacommunities and biodiversity patterns in Mediterranean temporary ponds:

the role of pond size, network connectivity and dispersal mode

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Metacommunities and biodiversity patterns in Mediterranean temporary ponds: the role of pond size, network connectivity and dispersal mode

IRENE TORNERO PINILLA

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DOCTORAL PROGRAMME IN WATER SCIENCE AND TECHNOLOGY

SUPERVISED BY

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Thesis submitted in fulfilment of the requirements to obtain the Degree of Doctor at the University of Girona

Universitat
de Girona

Dr Dani Boix Masafret and Dr Stéphanie Gascón Garcia, from the University of Girona,

DECLARE:

That the thesis entitled **Metacommunities and biodiversity patterns in Mediterranean temporary ponds: the role of pond size, network connectivity and dispersal mode** submitted by Irene Tornero Pinilla to obtain a doctoral degree has been completed under our supervision.

In witness thereof, we hereby sign this document.

Dr Dani Boix Masafret

Dr Stéphanie Gascón Garcia

Girona, 22nd November 2019

A mi familia

Caminante, son tus huellas
el camino y nada más;
Caminante, no hay camino,
se hace camino al andar.
Al andar se hace el camino,
y al volver la vista atrás
se ve la senda que nunca
se ha de volver a pisar.
Caminante no hay camino
sino estelas en la mar.

Antonio Machado

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LIST OF PUBLICATIONS DERIVED FROM THIS THESIS

Tornero, I., Sala, J., Gascón, S., Àvila, N., Quintana, X. D., & Boix, D. (2014). Aquatic macrofauna of Vila Nova de Milfontes temporary ponds, with the first record of *Cyphon hilaris* Nyholm, 1944 (Coleoptera: Scirtidae) from Portugal. *Boletín de la Sociedad Entomológica Aragonesa*, 55, 326-30.

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LIST OF ABBREVIATIONS AND ACRONYMS

-μ: body size diversity

-abs|100-%O|: oxygen absolute deviation from saturation

-aD%O: oxygen as absolute deviation (in percentage)

-AD: macrofaunal active dispersers

-AICc: Akaike's information criterion for small sample sizes

-ATD: Average taxonomic distinctness

-BD: total beta diversity

-BD-ad: abundance difference component of beta diversity

-BD-re: replacement component of beta diversity

-BS GM: body size geometric mean

-BS: body size

-Ch: chlorophyll-a

-Chla: Planktonic chlorophyll-a

-Clo: closeness

-Cond: conductivity

-Depth: maximum depth

-DIC: Dissolved inorganic carbon

-DIN: Dissolved inorganic nitrogen

-DNP: Distance to the nearest pond

-DOC: Dissolved organic carbon

-DW: dry weight

-ECELS: "Estat de conservació d'ecosistemes lenítics soms" (Shallow Lentic Ecosystem Conservation Status)

-H': Shannon-Wiener index

-IFO: Index of faunal originality

-LCBD: Local contribution to beta diversity

-LCBD-ad: abundance difference component of the Local contribution to beta diversity

-LCBD-re: replacement component of the Local contribution to beta diversity

-LEPN: Large extent pond network

-Macrophytes_DW and Macrop_DW: macrophytes biomass

-MTP: Mediterranean temporary ponds

-N: total nitrogen

-NBSS: normalised biomass-size spectra slope

-network id: network identity

-NMDS: Non-parametric multi-dimensional scaling

-NW: network

-P: total phosphorus

-PC: Principal component

-PCA: Principal component analysis

-PD: macrofaunal passive dispersers

-PDist: percolation distance

-PL: plants

-PO_4^{3-}: phosphate

-precip: precipitation

-PSSAM: percentage of species that presented a value of SCBD above the mean value of the sampled ponds

-S: taxa richness

-SCBD: Species contribution to beta diversity

-SCBD-prop: proportion between the number of species with a value of SCBD above the mean value of the set of sampled ponds in a network to the total species richness of the pond

-SEA: spatial extent measured as an area

-SED: spatial extent measured as a distance

-SEPN: Small extent pond network

-S-rar: rarefied species richness

-T: water temperature

-TD: Taxonomic distinctness

-temp: atmospheric temperature

-TIC: Total inorganic carbon

-TN: Total nitrogen

-TOC: Total organic carbon

-TP: Total phosphorus

-TRIX: Trophic index

-VM: Vila Nova de Milfontes

-VTD: Variation in taxonomic distinctness

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ABSTRACT

Understanding how ecological communities are structured is a fundamental question on ecology which is still unanswered. In this line, the role of environmental and spatial processes operating in multiple scales to shape local community composition is explicit in the metacommunity framework. Further, metacommunity dynamics are strongly dependent on three factors —scale, habitat heterogeneity and connectivity. Indeed, connectivity is considered an important attribute of natural ecosystems that sustains both biodiversity and ecosystem function. Moreover, there is evidence for variation in the spatial processes and patterns driving biodiversity at different spatial scales. Finally, habitat heterogeneity has been identified as a key factor for the distribution, abundance and diversity of species. Within this context, the aim of this thesis was to analyze how network connectivity, environmental characteristics and organisms' dispersal mode act in shaping macrofaunal metacommunities, taking into account different spatial scales. Pond networks constitute an ideal scenario to study assembly processes in the metacommunity framework, since for aquatic organisms, ponds are suitable patches in an unsuitable habitat matrix. Thus, ponds act as "habitat islands" and this particular "isolation" can, further, modulate the effect of both environmental and spatial factors on their communities. Moreover, pond networks are also good candidate ecosystems in which to examine the mechanisms underlying biodiversity patterns at local and regional scale since, on one hand, individual ponds constitute discrete systems suitable for studying their local environmental factors and communities, and, on the other hand, the whole network allows observing the dynamics at metacommunity level. Therefore, we selected and sampled four networks of Mediterranean temporary ponds covering a wide range of pond sizes and different spatial extents. Firstly, we studied the smallest pond network and we observed that pond size was not related to any of the environmental factors taken into account, and neither to macrofaunal community structure despite a positive relationship between pond size and for instance, species richness, was expected. However, pond size had an effect on community composition since certain species (such as *Gyraulus laevis*, *Piona* sp. and *Aeshna mixta*) had higher abundances in larger ponds. Moreover, the effect of a high connectivity throughout the network and therefore, a likely homogenization of the communities, seemed to explain the absence of significant differences in community structure. Secondly, we analyzed the effect of the spatial extent of the network comparing the decay of community similarities with distance (both spatial and environmental) among the ponds from two pond networks (one four times larger than the other), and also possible differences due to organisms' dispersal mode. In general, environmental distance had larger effects than spatial distance in both pond networks, probably due to the small extent of both networks. However, when carefully analyzing the responses observed, some differences arise linked to the spatial extent and the dispersal mode. Thus, in the smaller network, the different biotic groups showed different distance-decay patterns, that fit under different metacommunity archetypes: the mass effects in the case of the macrofaunal active dispersers, the species sorting for the macrofaunal passive dispersers, and undetermined for plants. In contrast, when increasing the spatial extent (i.e., in the larger network), a homogenization of the observed response occurred, and all the groups fit to the expected response under a species sorting archetype. Thirdly, we studied four pond networks applying the graph theory to obtain several connectivity network descriptors and centrality metrics. The network centrality metrics are descriptors of the degree of the isolation-centrality situation that a patch has with respect to the rest of the patches in the network. Then, we tried to identify if biodiversity patterns, at local and regional level, can be related to these centrality metrics. The environmental characteristics of the ponds, which included habitat condition, water characteristics and pond size, were also included in the analyses. We observed that the different centrality metrics, which measure connectivity within the network from a neighboring to a broader scale, affect both regional and local pond biodiversity. Moreover, most of the biodiversity metrics were not explained by environmental characteristics or network centrality metrics alone, but through a combination of them both. Overall, regional biodiversity metrics showed similar patterns in all the networks studied, whereas local biodiversity metrics showed greater network-dependent patterns. Therefore, considering the findings of this thesis, we argue that further studies on pond metacommunities at different spatial extents are needed and, especially, at small spatial extents, since there is a lack of works on metacommunities conducted at small scales, and we have demonstrated that the results can differ from those found in studies at larger scales, providing, then, new knowledge. Moreover, we demonstrated the effect on biodiversity of the connectivity within a pond network, highlighting the importance of preserving habitat connectivity. On the other hand, although we did not find support for the expected positive species-area relationship, we found differences at composition level. Thus, we think that it is important to maintain size diversity of ponds, since smaller waterbodies do not seem to harbour less species than larger ponds but, certain species are favoured in smaller ponds whereas other species are favoured in larger ones. Moreover, smaller ponds can further, act as stepping-stones helping to maintain metacommunity dynamics and stability.

RESUMEN

Entender cómo están estructuradas y cómo se estructuran las comunidades ecológicas es una de las preguntas fundamentales de la ecología que está aún sin resolver. En este sentido, el papel de los procesos ambientales y espaciales que operan a múltiples escalas para conformar la composición de la comunidad a nivel local está explícito en el marco teórico de la metacomunidad. Además, las dinámicas de la metacomunidad dependen ampliamente de tres factores —la escala, la heterogeneidad del hábitat y la conectividad. De hecho, la conectividad se considera una característica de los ecosistemas naturales que mantiene tanto la biodiversidad, como el funcionamiento del ecosistema. Asimismo, existen evidencias de un efecto de la escala espacial respecto a los procesos y patrones que modifican los patrones de la biodiversidad. Finalmente, la heterogeneidad del hábitat ha sido identificada como un factor clave para la distribución, la abundancia y la diversidad de especies. En este contexto, el objetivo de esta tesis era analizar cómo el tamaño de las lagunas, la conectividad de la red, las características ambientales y el modo de dispersión de los organismos actúan modelando las metacomunidades de macrofauna, considerando además, diferentes escalas espaciales. Las redes de lagunas constituyen un escenario ideal para estudiar los procesos a nivel de metacomunidades, ya que, para los organismos acuáticos, las lagunas suponen islas de hábitats favorables para ellos que están inmersas en una matriz constituida por un hábitat que les resulta desfavorable. Por lo tanto, las lagunas actúan como "islas de hábitat" y esta particular condición de "aislamiento" puede, además, modular el efecto en sus comunidades tanto de los factores ambientales como de los espaciales. Además, las redes de lagunas son también buenos ecosistemas candidatos en los que examinar los mecanismos subyacentes a los patrones de biodiversidad tanto a escala local como regional, puesto que, por un lado, cada laguna individualmente constituye un sistema adecuado para estudiar los factores ambientales locales y sus comunidades, y por otro, el conjunto de la red permite observar las dinámicas a nivel metacomunitario. Para ello seleccionamos y muestreamos cuatro redes de lagunas temporales mediterráneas de diferentes extensiones y con un rango amplio de tamaños de lagunas. En primer lugar, estudiamos la red más pequeña y observamos que el tamaño de la laguna no estaba relacionado con ninguno de los factores ambientales que habíamos tenido en cuenta, ni con la estructura de la comunidad de macrofauna, a pesar de que esperábamos encontrar una relación positiva entre el tamaño de la laguna y, por ejemplo, la riqueza de especies. Sin embargo, el tamaño de la laguna produjo un efecto en la composición de la comunidad ya que ciertas especies (como *Gyraulus laevis*, *Piona* sp. y *Aeshna mixta*) presentaron mayores abundancias en las lagunas más grandes. Además, el

efecto producido por una alta conectividad dentro de la red y, en consecuencia, la probable homogeneización de las comunidades, parece explicar la ausencia de diferencias significativas en la estructura de la comunidad. En segundo lugar, analizamos el efecto de la extensión espacial de la red al analizar la pérdida de similitud en las comunidades entre las lagunas como consecuencia de la distancia (tanto espacial como ambiental) comparando dos redes (una es cuatro veces más grande que la otra) y analizamos también las posibles diferencias debidas al modo de dispersión de los organismos. En general, en ambas redes la distancia ambiental tuvo más efecto que la distancia espacial debido probablemente a la pequeña extensión de las dos redes. Aunque cuando analizamos en detalle las respuestas observadas, encontramos algunas diferencias relacionadas con la extensión de la red y el modo de dispersión de los organismos. Así, en la red más pequeña, los diferentes grupos bióticos presentaron diferentes patrones de pérdida de similitud con la distancia que pueden encajar en los diferentes arquetipos de la metacomunidad: el ‘efecto de masas’ en el caso de los dispersores activos de la macrofauna, el ‘filtro de las especies’ en el caso de los dispersores pasivos, y un patrón indeterminado para las plantas. Por el contrario, cuando aumentaba la extensión espacial (es decir, en la red más grande), la respuesta observada era más homogénea, y correspondería a la situación esperada cuando el arquetipo mayoritario es el ‘filtro de las especies’. En tercer lugar, estudiamos las cuatro redes de lagunas aplicando la teoría de grafos para obtener diversos descriptores y métricas de centralidad de la red. Las métricas de centralidad de la red son descriptores del grado de aislamiento-centralidad de un punto de la red respecto al resto de puntos. Con esto intentamos identificar si los patrones de biodiversidad tanto a nivel local como regional pueden relacionarse con estas métricas de centralidad. También se incluyeron en los análisis las características ambientales de las lagunas, considerando dentro de éstas el estado de conservación del hábitat, las características del agua y el tamaño de la laguna. Observamos que las diferentes métricas de centralidad, que miden la conectividad dentro de la red desde una escala local hasta una escala más amplia, afectan tanto a la biodiversidad regional como local de las lagunas. Además, la mayoría de las métricas de biodiversidad no se explicaron solo por las características ambientales o las métricas de centralidad, sino por la combinación de ambos factores. En general, las métricas de biodiversidad regional mostraron patrones similares en todas las redes estudiadas, mientras que las métricas de biodiversidad local mostraron patrones más dependientes del tipo de red. Por lo tanto, los resultados de esta tesis indican que es necesario llevar a cabo más estudios sobre metacomunidades de lagunas considerando diferentes extensiones espaciales y, especialmente, a escalas pequeñas, ya que existen pocos estudios sobre metacomunidades en estas escalas, a pesar de que, como

hemos demostrado, los resultados pueden diferir de aquéllos llevados a cabo en escalas mayores, aportando, por tanto, nuevo conocimiento en este tema. Asimismo, hemos demostrado el efecto en la biodiversidad de la conectividad dentro de una red de lagunas, remarcando la importancia de conservar la conectividad. Por otra parte, aunque no hemos encontrado evidencias que apoyaran la esperada relación entre las especies y el área, sí que encontramos diferencias a nivel de composición de la comunidad. Por todo ello, consideramos que es importante mantener la diversidad de tamaños de lagunas ya que no parece que las masas de agua más pequeñas alberguen una menor riqueza de especies que las más grandes, y por el contrario, ciertas especies se ven favorecidas en lagunas más pequeñas y otras especies en lagunas más grandes. Además, las lagunas de menor tamaño pueden, a su vez, actuar como pasos intermedios hacia otras lagunas, ayudando así a mantener las dinámicas y la estabilidad de la metacomunidad.

RESUM

Entendre com estan estructurades i com s’estructuren les comunitats ecològiques és una de les preguntes fonamentals de l'ecologia i és un tema d’actualitat en la recerca científica, ja que nous marcs teòrics han propiciat un avenç significatiu en el seu coneixement. En aquest sentit, el marc teòric que suposa el concepte de metacomunitat explicita el paper dels processos ambientals i espacials que operen a múltiples escales per conformar la composició de la comunitat a nivell local. A més, les dinàmiques de la metacomunitat es veuen alterades per tres factors —l’escala espacial, l'heterogeneïtat de l'hàbitat i la connectivitat. De fet, la connectivitat es considera una característica determinant de la biodiversitat i del funcionament dels ecosistemes naturals . Així mateix, hi ha evidències d’un efecte de l’escala espacial pel que fa als processos que modifiquen els patrons de la biodiversitat. Finalment, l’heterogeneïtat dels hàbitats s’ha identificat com un factor clau per a la distribució, l’abundància i la diversitat d’espècies. En aquest context, l'objectiu d'aquesta tesi era analitzar com la mida de les llacunes, la connectivitat de la xarxa, les característiques ambientals i el tipus de dispersió dels organismes actuen modelant les metacomunitats de macrofauna, considerant a més diferents escales espacials. Les xarxes de llacunes constitueixen un escenari ideal per estudiar processos a nivell de metacomunitats ja que, per als organismes aquàtics, les llacunes suposen illes d’habitats favorables que es troben dins d’una matriu constituïda per un hàbitat que els és desfavorable. Per tant, les llacunes actuen com a "illes d'hàbitat" i aquesta particular condició de "aïllament" pot, a més, modular l'efecte en les seves comunitats tant dels factors ambientals com dels espacials. A més, les xarxes de llacunes són també ideals per examinar els mecanismes subjacents als patrons de biodiversitat tant a escala local com regional, ja que, d'una banda, cada llacuna individualment constitueix un sistema adequat per a estudiar els factors ambientals locals i com afecten a les seves comunitats, i d'altra, el conjunt de la xarxa permet observar les dinàmiques a nivell metacomunitari. Per això, s’han seleccionat i mostrejat quatre xarxes de llacunes temporànies mediterrànies de diferents extensions i amb un rang ampli de mides de llacunes. En primer lloc, es va estudiar la xarxa més petita i es va observar que la mida de la llacuna no estava relacionada amb cap dels factors ambientals que s’havien considerat, ni amb l'estructura de la comunitat de la macrofauna, tot i que esperàvem trobar una relació positiva entre la mida de la llacuna i, per exemple, la riquesa d'espècies. No obstant això, la mida de la llacuna si que es va relacionar amb la composició de la comunitat ja que certes espècies (com *Gyraulus laevis*, *Piona* sp. i *Aeshna mixta*) van presentar majors abundàncies en les llacunes més grans. A més, l'efecte produït per una alta connectivitat dins de la xarxa, i en

conseqüència, la probable homogeneïtzació de les comunitats, podria explicar l'absència de diferències significatives en l'estructura de la comunitat. En segon lloc, vam analitzar la relació entre la similitud de les comunitats i la distància, tant espacial com ambiental. A més, es va considerar també un possible efecte de l'extensió espacial de la xarxa tot comparant dues xarxes (essent una quatre vegades més gran que l'altra). Alhora, també es va analitzar possibles diferències degudes al tipus de dispersió dels organismes. En general, en totes dues xarxes la distància ambiental va tenir més efecte que la distància espacial degut probablement a la petita extensió de les xarxes estudiades. Tot i així, a l'analitzar en detall les respostes observades, es van detectar diferències relacionades amb l'extensió de la xarxa i el tipus de dispersió dels organismes. Així, a la xarxa més petita, els diferents grups biòtics van presentar diferents patrons de pèrdua de similitud amb la distància que encaixen amb diferents arquetips de metacomunitat: l' 'efecte de masses' en el cas dels dispersors actius de la macrofauna, el 'filtratge d'espècies' en el cas dels dispersors passius, i un patró indeterminat per a les plantes. Per contra, a l'augmentar l'extensió espacial (és a dir, a la xarxa més gran), la resposta observada era més homogènia entre els diferents grups biòtics, i corresponia a la situació esperada quan l'arquetip dominant és el 'filtratge d'espècies'. En tercer lloc, es va analitzar quatre xarxes de llacunes aplicant la 'teoria de grafs' per obtenir diversos descriptors i mètriques de centralitat de la xarxa. Les mètriques de centralitat de la xarxa són descriptors del grau d'aïllament-centralitat d'un punt de la xarxa pel que fa a la resta de punts. Així, es pretenia identificar si els patrons de biodiversitat, tant a nivell local com regional, poden relacionar-se amb aquestes mètriques de centralitat. També es van incloure en les anàlisis les característiques ambientals de les llacunes, considerant dins d'aquestes l'estat de conservació de l'hàbitat, les característiques de l'aigua i la mida de la llacuna. Es va observar que les diferents mètriques de centralitat, que mesuren la connectivitat dins de la xarxa des d'una escala local fins a una escala més àmplia, afecten tant a la biodiversitat regional com local de les llacunes. A més, la majoria de les mètriques de biodiversitat no van ser explicades només per les característiques ambientals o les mètriques de centralitat, sinó per la combinació d'ambdues. En general, les mètriques de biodiversitat regional van mostrar patrons similars en totes les xarxes estudiades, mentre que les mètriques de biodiversitat local van mostrar patrons més dependents del tipus de xarxa. Per tant, els resultats d'aquesta tesi, subratllen la necessitat de dur a terme més estudis sobre metacomunitats de llacunes considerant diferents extensions espacials i, especialment, en escales petites, ja que s'han observat diferencies respecte als patrons observats en xarxes de major extensió, aportant, per tant, nou coneixement en aquesta matèria. També s'ha demostrat l'efecte de la connectivitat dins d'una xarxa de llacunes sobre la biodiversitat,

remarcant la importància de conservar la connectivitat. D'altra banda, tot i que no hem trobat evidències que recolzessin l'esperada relació entre el nombre d’espècies i la mida de l’ecosistema, sí que hem trobat diferències a nivell de composició de la comunitat. Per tot això, és important mantenir la diversitat de mides de llacunes, ja que no sembla que les masses d'aigua més petites tinguin una menor riquesa d'espècies que les més grans, i per contra, certes espècies es veuen afavorides en llacunes més petites i altres espècies en llacunes més grans. A més, les llacunes més petites poden, al seu torn, actuar com a passos intermedis cap a altres llacunes, ajudant així a mantenir les dinàmiques i l'estabilitat de la metacomunitat.

1. INTRODUCTION

1.1. Mediterranean temporary ponds

Despite they have been undervalued for so long (De Meester et al., 2005; Boix et al., 2012; Jeffries et al., 2016), ponds present a high value regarding their biodiversity and socio-economic benefits (Oertli et al., 2004; Céréghino et al., 2008; EPCN, 2008). Considered as biodiversity hotspots, ponds support a wide range of rare and endangered plants and animals. Moreover, they provide several ecosystem services such as acting as stepping stones for migration (Merriam, 1991; Gibbs, 1993; Incagnone et al., 2015), dispersal and genetic exchange of species (Mushet et al., 2013; Céréghino et al., 2014), which is particularly important in the context of climate change (EPCN, 2008; Calhoun et al., 2017). Single ponds can act as refuges for both terrestrial and aquatic organisms (e.g., Boix et al., 2001; Lott, 2001; Strachan et al., 2014; Mushet et al., 2019), but networks of ponds are critical to support the metapopulations of many species, to the conservation of amphibians, as fish habitats or even for wetland mammals and birds that need ponds as part of a complex mosaic of the wetlands they utilize (e.g., Gibbs, 1993; Paton 2005; EPCN, 2008; Mushet et al., 2013). Therefore, despite their isolated nature, they perform an important ecological role at the landscape level, for instance in a metacommunity context (Jeffries 1994; Céréghino et al., 2014) since for obligatory aquatic organisms, ponds are suitable patches in an unsuitable habitat matrix (De Meester et al., 2005). For many years, small ecosystems as freshwater ponds and pools have been less appreciated and attended than larger ecosystems such as marine systems, lakes, rivers or coral reefs and even their abundance was underestimated (Downing et al., 2006). However, despite being small ecosystems, they greatly contribute to regional diversity mainly due to their high beta diversity (i.e., compositional dissimilarity among sites) (Oertli et al., 2002; Williams et al., 2003; De Meester et al., 2005; Mushet et al., 2019).

Despite the proven benefits that they produce, ponds have received little effective protection from legislation (Céréghino et al., 2014; Boix et al., 2016; Calhoun et al., 2017). Especially forgotten for decades have remained the temporary ponds, which are commonly extended throughout the entire world (Jeffries et al., 2016; Calhoun et al., 2017; Boix et al., 2019), but are a particularly important pond type in arid and semi-arid regions as the Mediterranean basin (Williams, 1985; Williams, 2006; Boix et al., 2016). Since they support threatened and endemic species (Zedler, 2003; Boix et al., 2016), Mediterranean temporary ponds (MTP) are protected under the European Commission's Habitat Directive 92/43/EEC. MTP are especially vulnerable since they are shallow and often small in area and volume, which makes them susceptible to anthropogenic threats such as pollution, drainage and destruction (EPCN, 2008; Grillas et al., 2004; Boix et al., 2016). MTP are flooded in winter or in the end of spring and dry out in summer. During the flooding phase they harbor species (both from fauna and flora) that clearly differ from the ones that can be found in permanent ponds, and are, in some cases, species of great conservation importance (Collinson et al., 1995; Zacharias & Zamparas, 2010; Boix et al., 2016). This means that only very specific biota inhabit this type of systems since the species may require specific adaptations to deal with variability and extremity of the environmental conditions, including time stress for development and reproduction, and mechanisms to span dry periods (Wiggins et al., 1980; King et al., 1996; De Meester et al., 2005; Zacharias et al., 2007). Due to the hydrological changes produced during the hydroperiod, changes in the patterns and strength of biotic interactions may also occur. These natural changes cause, in turn, the change of the aquatic vegetation and the invertebrate fauna (Boix et al., 2004; Florencio et al., 2009; Sahuquillo & Miracle, 2010).

1.2. Size matters: pond size and network spatial extent

The spatial scale of ecological data encompasses both local (i.e., pond) and regional level (the spatial extent of the pond network). 'Pond size' is the first level of spatial resolution possible with a given data set, whereas 'spatial extent' is the total area of the study (Turner et al., 1989; Scheiner et al., 2000; Whittaker et al., 2001; Willig et al., 2003). When sampling species information, it must be kept in mind that the number of species in a sample might be influenced by both pond size and the spatial extent of the pond network (Wiens, 1989; Palmer & White, 1994; Dungan et al., 2002). Therefore, the extent of an area or gradient sampled can have for instance, a pronounced impact on the derived pattern of species richness. Moreover, we have to add to this fact the complication that the influence of range size distribution on species richness patterns is related to the spatial scale at which different taxa perceive the environment, according to their body size and/or dispersal capabilities (Rahbek, 2005). Thus, it is highly likely that organisms with different dispersal modes will present patterns of beta diversity which respond differently to changes in spatial extent (Barton et al., 2013). For instance, when evaluating the effect of increasing the scale of beta diversity from a local to a regional scale, dispersal limitation or metacommunity dynamics will become determinant since different environmental factors will also acquire importance. The study of community assemblages in temporary waters can add a particularly interesting view since these habitats can be considered as 'habitat islands' and therefore, the different predictions of the island biogeography theory (MacArthur & Levins, 1967; Ebert & Balko, 1987; March & Bass, 1995; Spencer et al., 1999), the island paradigm (Weiher & Keddy, 1999), and the metapopulation dynamics theory (Levins, 1969; Hanski & Gilpin, 1991) can be tested on them. Thus, in order to test one of the principles of the island biogeography theory that states the existence of a relationship between the size of an island and its species richness, ponds (including both temporary and permanent ones) have often been subject of study of the species-area relationship, including a wide range of organisms with different dispersal abilities (e.g., Friday, 1987; King et al., 1996; Gee et al., 1997; Oertli et al., 2002; Reche et al., 2005). However, the type of relationship specifically in temporary ponds is not yet clear since either a positive relationship or an absence of relationship have been found (Ebert & Balko, 1987; Bilton et al., 2001b; Brose, 2001; Della Bella et al., 2005). Besides, only few of the existent works have analysed the effect of pond area considering the entire macroinvertebrate community (Gee et al., 1997).

On the other hand, several studies have also highlighted the strong scale-dependency of the patterns in beta diversity (e.g., Soininen et al., 2007a; Declerck et al., 2011; Barton et al., 2013; Heino et al., 2015b; Zorzal-Almeida et al., 2017; Sreekar et al., 2018) and in alpha diversity (e.g., Gering & Crist, 2002; Sreekar et al., 2018; Chase et al., 2019). Additionally, the distance-decay of community similarity (Nekola & White, 1999), which is an approach to study spatial variation in beta diversity, can be also modulated when the spatial extent of the study area varies. Generally, the rate of decay is higher (i.e., steeper slope) at smaller study extents and lower at larger extents (Steinitz et al., 2006; Soininen et al., 2007b; Steinbauer et al., 2012). Thus, it will be interesting to consider the likely effects that both the pond size and the spatial extent of the pond network can have on community assembly. Moreover, studying pond networks with different spatial extents can be interesting to observe possible different patterns in biodiversity, considering both alpha and beta diversity.

1.3. Spatial versus environmental factors and the effects on the organisms according to their dispersal mode

The identification of the mechanisms driving variation in, and among, local communities is central to community ecology. The role of environmental and spatial processes operating in multiple scales to shape local community composition is explicit in the metacommunity framework (Leibold et al., 2004, 2017; Stendera et al., 2012). Several local environmental factors but also factors acting at multiple spatial scales have been identified as determinants of local communities (Ricklefs & Schluter, 1993). Cottenie (2005) reviewed more than 150 data sets on community structure and showed that almost 50% of the variation in community composition is explained by both environmental and spatial variables. In ponds, many factors have been pointed as natural drivers determining biodiversity and community structure, and in many cases, it was the combination of several factors the responsible for biodiversity patterns (Stendera et al., 2012). Within environmental factors, some of them act at local level, whereas others at regional level. Some of the environmental local factors are, for instance, macrophytes density, habitat area, water chemistry and trophy level, and some of the environmental regional factors are, for instance, geology, history and climate. Moreover, aquatic organisms with different dispersal modes may show different regional versus local environmental influences in community composition (Padial et al., 2014; Heino et al., 2017) and so, there are different landscape perceptions among different taxa which will, in turn, affect local diversity patterns (Borthagaray et al., 2015a). Metacommunity theory provides a framework to describe the underlying environmental and spatial processes influencing community composition and beta diversity (Cottenie, 2005; Grönroos et al., 2013). Thus, when focusing on metacommunities, the structures of communities may differ depending on their locations within a landscape even when local patch conditions (e.g., area or heterogeneity) are similar among locations (Economo & Keitt, 2008, 2010; Logue et al., 2011). Therefore, analysing the role of environmental and spatial factors can help us to understand which type of metacommunities we are finding, which metacommunity archetypes might be structuring our communities and whether these factors interact and influence the relative contribution of each component to the total beta diversity.

1.4. Connectivity and pond network structure in a metacommunity context

The knowledge on the processes shaping community assembly has improved thanks to the metacommunity concept since it emphasizes the interdependence of local communities and the regional species pool (Holyoak et al., 2005). Since the beginning of the syntheses by Leibold et al. (2004) and Holyoak et al. (2005) of the metacommunity idea as a result of the Metacommunity Working Group at NCEAS until the present, several changes have happened and the theory has evolved. Even the original authors themselves have stated their embrace towards gaining new complementary insights about different aspects of metacommunity dynamics from new studies covering from observational and experimental approaches, to analytical methods (Leibold & Chase, 2018). The main novelty introduced in the theory over the years according to its own authors is the change from a compartmentalized view of the four "paradigms" (i.e., species sorting, patch dynamics, neutral models and mass effects dynamics; (Leibold et al., 2004; Holyoak et al., 2005) to the necessity of a more pluralistic and nuanced perspective of what they renamed now "archetypes" (Leibold & Chase, 2018). Moreover, these authors admit that they "do not have the right tools that can fully deal with the complexity of metacommunity dynamics or at least not by themselves". According to Leibold & Chase (2018), three main mechanisms —dispersal, trait-by-environment matching and stochastic ecological drift— encapsulate the metacommunity ecology and constitute the core of the different metacommunity archetypes previously mentioned. In turn, these three mechanisms are, on one hand, strongly modified by three factors —scale, habitat heterogeneity and connectivity— that at last influence the relative importance of the different mechanisms and, on the other hand, by evolutionary processes and trophic interactions. Therefore, throughout this thesis some of these mechanisms and factors acquire great prominence. Connectivity is especially important in the context of this thesis given that we will study the metacommunities of networks encompassed by ponds and other water bodies, which constitute patches that are distributed over a terrestrial matrix. In Ecology, connectivity has been considered an emergent property of landscapes encapsulating individuals' flows across space (Baguette et al., 2013). Concretely, landscape connectivity is the degree to which the landscape facilitates or impedes movement among resource patches and the term includes both 'structural connectivity' (i.e., the physical relationships between habitat patches) and 'functional connectivity' (i.e., an organism's behavioural response to both the landscape structure and the landscape matrix) (Taylor et al., 1993; Borthagaray et al., 2014, 2015a). Connectivity is considered an important attribute of natural ecosystems that sustains both biodiversity and ecosystem function (Matisziw & Murray, 2009; Staddon et al., 2010). Hence, both increasing and decreasing landscape connectivity can generate changes in species diversity and ecosystem processes, depending on the initial level of landscape connectivity and the dispersal abilities of the organisms involved (Loreau et al., 2003b). Traditionally, the relative isolation of a community within a landscape is thought to have great influence on local diversity. Habitat isolation is determined by the distance to the nearest patch and the amount of habitat within an appropriate distance of the sampled patch (Fahrig, 2013). Therefore, the habitat in the landscape surrounding a patch is its primary source of colonists, so less individuals and species colonize a more isolated patch, reducing its species richness compared to a less isolated patch (Fahrig, 2013). Nevertheless, it is still difficult to quantify isolation in metacommunities with complex spatial structure (Economo & Keitt, 2010). One of the most used approaches in Ecology to represent spatially complex landscapes is the use of the graph theory (Keitt et al., 1997; Urban & Keitt, 2001; Bodin & Norberg, 2007; Estrada & Bodin, 2008; Minor & Urban, 2008; Urban et al., 2009; Borthagaray et al., 2015b) and it has been notably recommended for the study of metacommunities (Gonzalez et al., 2011; Altermatt, 2013). Graph theory supplies a great set of tools for representing metacommunities and quantifying their structures at the level of the whole network and at the level of individual communities. Thus, a metacommunity can be represented by a graph defined as a set of nodes connected by links (Borthagaray et al., 2015b). Once the graph is obtained, the 'network centrality measures' can be calculated to quantify local and regional node position in landscape networks (Estrada & Bodin, 2008; Economo & Keitt, 2010; Borthagaray et al., 2015). In addition to the numerous applications that graph theory can have for studying biodiversity in networks, it also offers a great potential for the management and conservation of freshwater ecosystems (Bunn et al., 2000; Estrada & Bodin, 2008; Matisziw & Murray, 2009).

So far, most of the studies applying graph theory to landscape networks were on metacommunities in dendritic (the theoretical ones) or river networks (the empirical ones) (e.g., Economo & Keitt, 2010; Erős et al., 2012; Seymour & Altermatt, 2014; Henriques-Silva et al., 2019), and only few in networks of lentic waterbodies (Ribeiro et al., 2011; Borthagaray et al., 2015a; Thornhill et al., 2018). Besides, the majority of the mentioned studies were theoretical or experimental (Economo & Keitt, 2010; Erős et al., 2012; Seymour & Altermatt, 2014) and few of them were using observational data (but see Ribeiro et al., 2011; Borthagaray et al., 2015; Henriques-Silva et al., 2019). On the other hand, few studies have tried to detect whether the spatial configuration of connections between communities (i.e., the network structure) can be an important determinant of community structure (Brown & Swan, 2010) although previous studies found that varying the spatial structure of the network affected both alpha and beta diversity (Economo & Keitt, 2008). Then, it will be interesting to apply graph theory to observational data from pond networks with different structures and therefore, different degrees of connectivity, in order to analyse their biodiversity patterns.

2. HYPOTHESES AND OBJECTIVES

Within the framework of this thesis, the hypotheses considered were:

1. The values of several biodiversity metrics are known to be influenced by both the size of the sampling unit and the extent of the studied area (Whittaker et al., 2001; Rahbek, 2005; Chase & Knight, 2013; Patrick & Yuan, 2019). Therefore, we expect that both pond size and the spatial extent of the pond network would explain, at least partially, the metacommunity composition and the biodiversity patterns of the aquatic communities (Figure 2.1) (**Chapter 1, 2 and 3**).

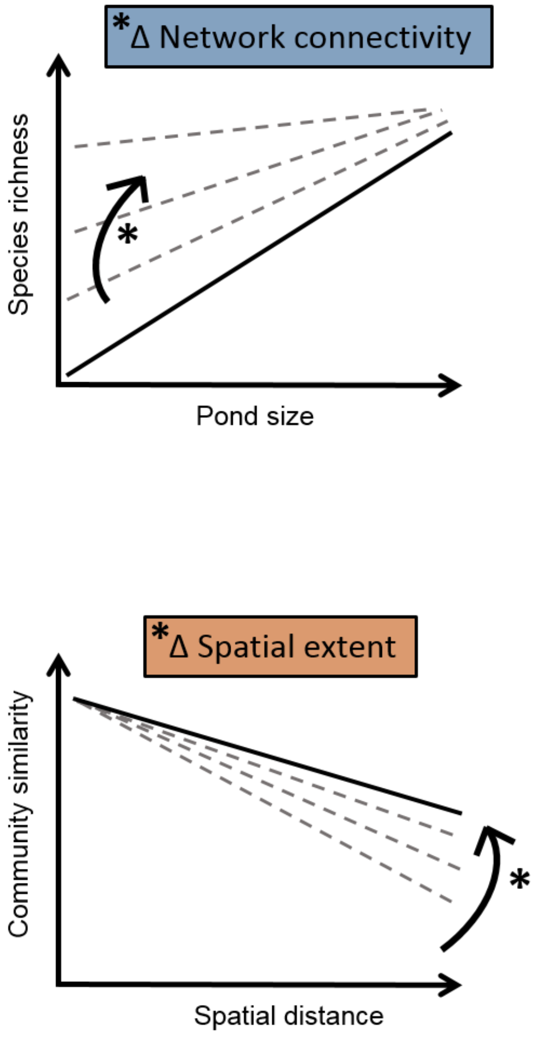

Figure 2.1. Conceptual diagram representing the hypothesis 1.

2. Both spatial and environmental factors affect the patterns of variation among local communities, which can alter local community processes that feed back to alter the regional biota (Leibold et al., 2004). The relative importance of both types of factors (spatial versus

environmental ones) has been related to dispersal capacity (Heino et al., 2014; Tonkin et al., 2016b). When studying metacommunities in relatively small spatial extents, dispersal rates are intermediate and do not homogenize community structure but allow species to track variation in environmental heterogeneity across sites. In this situation, we would expect a greater effect of environmental over spatial factors on community. In contrast, when connectivity is high, we would expect a greater effect of spatial factors since sites close to each other are homogenized to some degree by dispersal, and thus explaining a minor effect of environmental factors. Moreover, we also expect that the relative importance of spatial versus environmental factors on metacommunity assembly will be related to the dispersal mode of the organisms. Thus, active dispersers (due to their capability to decide where to go) would be more related to environment than passive dispersers, being the latter more related to spatial variables (Figure 2.2) (**Chapter 2**).

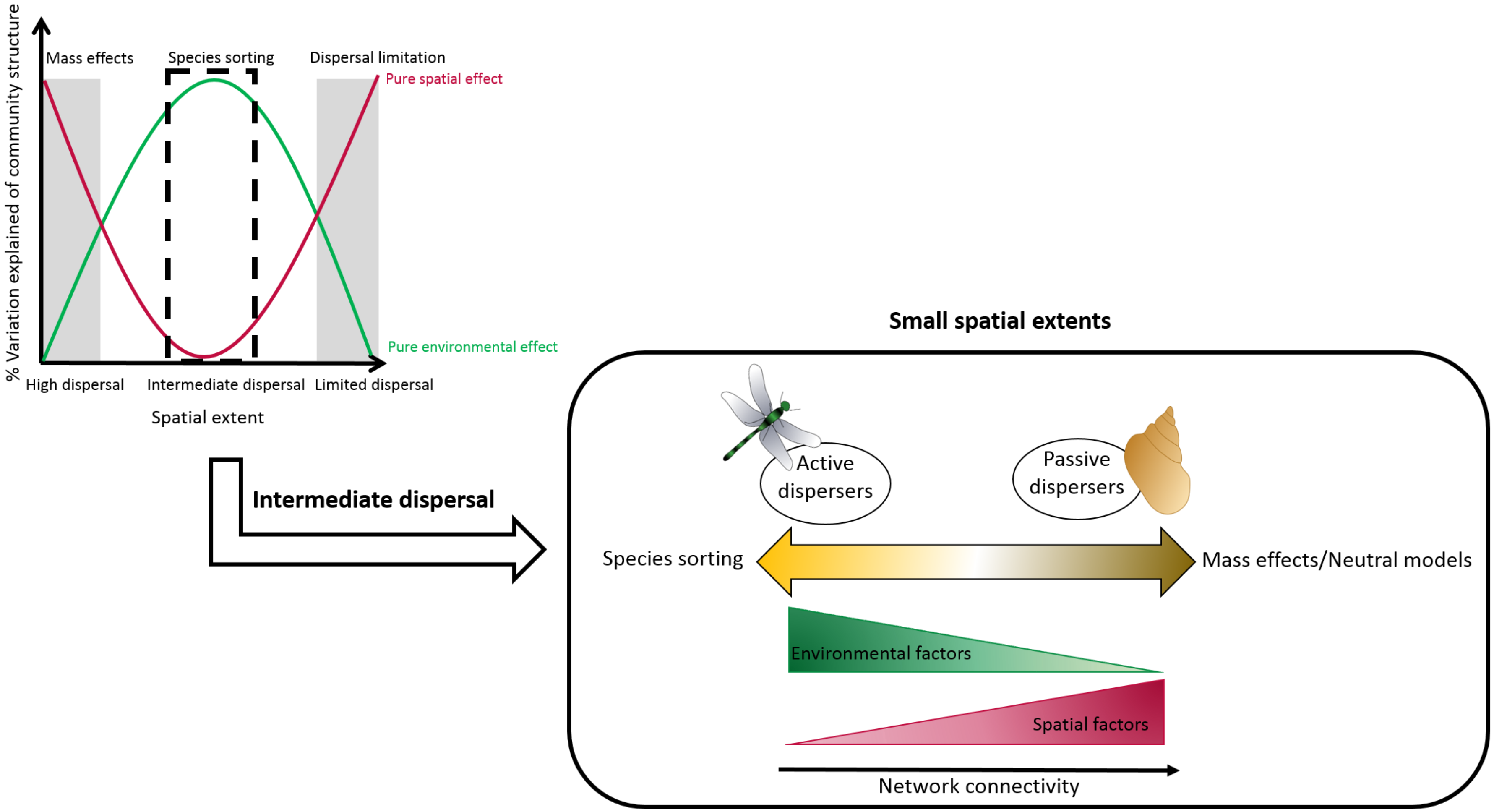

Figure 2.2. Conceptual diagram representing the hypothesis 2. The left upper part of the figure is modified from Heino et al., 2014.

3. According to the existing theoretical and empirical framework (Chase, 2003; Economo & Keitt, 2010; Borthagaray et al., 2015b), when analyzing networks of waterbodies with a gradient of connectivity within the network, we would expect that if connectivity is very high, the total beta diversity would decrease and the alpha diversity would increase in the more connected patches. Contrarily, if connectivity is very low, the total beta diversity would increase and the alpha diversity would decrease. Moreover, we expect that the gradient of isolation-centrality that a patch has with respect to its situation within the network would have an effect on the alpha diversity and would eventually also have an effect on the beta diversity (Figure 2.3) (**Chapter 3**).

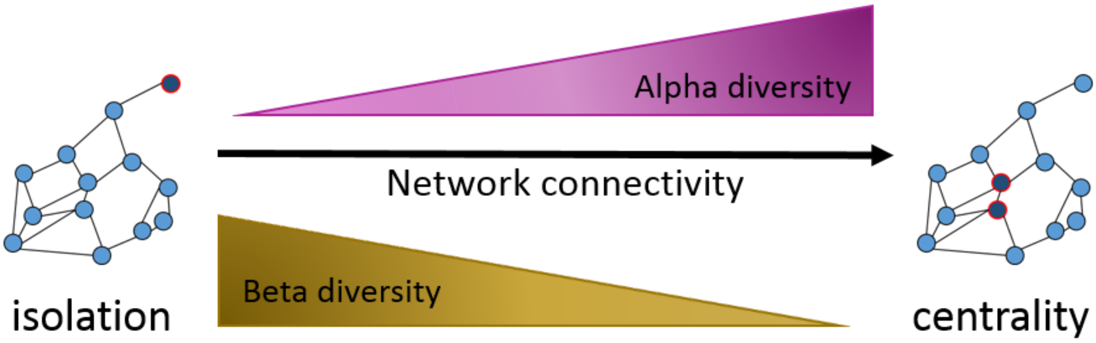

Figure 2.3. Conceptual diagram representing the hypothesis 3.

Therefore, taking into account these hypotheses, the general objective of this thesis is to analyze how network connectivity, environmental characteristics and organisms dispersal mode act in shaping macrofaunal metacommunities of Mediterranean temporary ponds, taking into account different spatial scales.

Concretely, the specific objectives for the three chapters of this thesis are:

- **1.** To test the effect of pond size on the composition and structure of the macrofaunal community and to analyze if other environmental factors could modulate the effects of pond size on the community (**Chapter 1 and 3**).
- **2.** To test the relative role of environmental versus spatial factors as drivers of community assembly (**Chapter 2 and 3**).
- **3.** To propose a new framework linking distance-decay patterns to different metacommunity dynamics taking into account both the dispersal mode of the organisms and the spatial extent of the study site (**Chapter 2**).
- **4.** To examine how the network structure and the connectivity within the network determines patterns of local and regional biodiversity in metacommunities taking also into account the potential role of local factors such as pond size, habitat quality, and environmental variability (**Chapter 3**).

3. GENERAL METHODOLOGY

3.1. Study sites

Throughout this thesis, 45 temporary ponds distributed in 4 different sites were studied. All of them are included in protected natural areas within the Mediterranean area. In each site, a pond network encompassing a set of ponds (between 10 and 12) was selected for the study. Of the 4 pond networks, 2 of them were in Spain [Albera (hereafter NW 1) and Guils de Cerdanya (hereafter NW 2)], 1 was in Portugal [Vila Nova de Milfontes (hereafter NW 3)], and 1 was in Italy [Giara di Gesturi (hereafter NW 4)] (Figure 3.1).

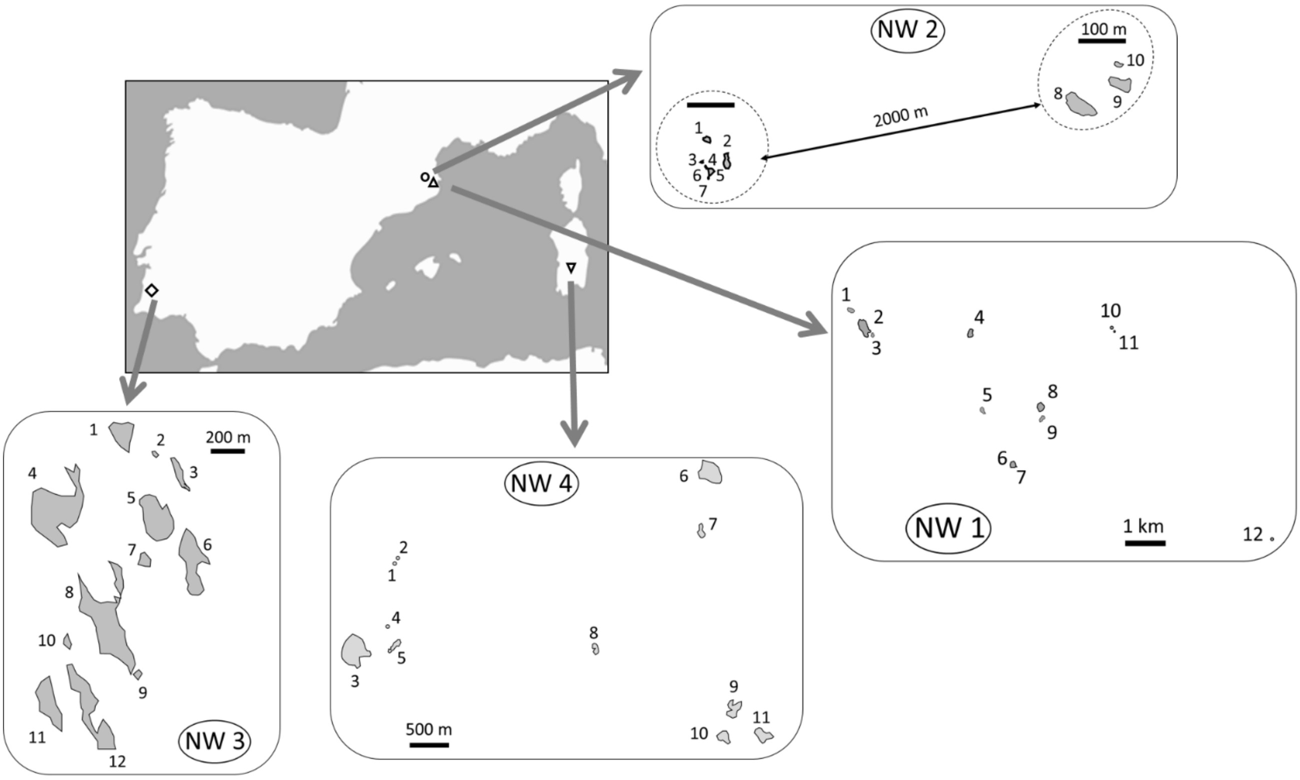

Figure 3.1. Map of the study sites indicating the ponds sampled in each network. NW 1 corresponds to the pond network in Albera (NE Spain), NW 2 to Guils de Cerdanya (NE Spain), NW 3 to Vila Nova de Milfontes (SW Portugal) and NW 4 to Giara di Gesturi (Sardinia, Italy).

The twelve temporary ponds from the NW 1 are located within the lowlands of the Albera massif (42°22’ N 2°57’ E) (eastern Pyrenees) which is situated within the Natural Zone of Declared National Interest of the Albera, under the Directive 92/43/CEE. The ponds are placed at 200 m altitude, and the area consists mainly of granites and schists. The soils are siliceous and relatively acid. These ponds usually flood with rainwater during the wet seasons (especially in autumn and/or spring) (Ruhí et al., 2012; Compte et al., 2016; Cunillera-Montcusí et al., 2019). The area is included within the ‘Csa’ Köppen-Geiger climate classification, which means that the climate is warm temperate, with hot and dry summers (Kottek et al., 2006) (Table 3.1 and Figure 3.2).

The ten temporary ponds from the NW 2 are close to the village of Guils de Cerdanya (42°28’ N 1°49’ E) within the Cerdanya-Alt Urgell National Hunting Reserve. The ponds are located within the Malniu-Guils glacial cirque, at approximately 2100 m altitude. The soils are siliceous (Palacios et al., 2015). The area is included within the ‘Cfb’ Köppen-Geiger climate classification, which means that the climate is warm temperate, fully humid and has warm summers (Kottek et al., 2006). The rainfall is mostly abundant in spring and autumn, and in winter, snow is present regularly (Vide & Olcina, 2001) (Table 3.1 and Figure 3.2).

The twelve temporary ponds sampled in NW3 are located at sea level, near the village of Vila Nova de Milfontes (37°45' N 8°48' W), within the Southwest Alentejo and Vicentine Coast Natural Park. The ponds are included under a Special Area of Conservation for the Natura 2000 Network (PTCON0012). They are situated on a coastal sandy plateau protected by consolidated dunes on the west, and by a wooded area to the east (Caramujo & Boavida, 2010). The area is included within the ‘Csa’ Köppen-Geiger climate classification, which means that the climate is warm temperate, with hot and dry summers (Kottek et al., 2006). The soil is highly permeable and ponds fill mainly with rainwater (Caramujo & Boavida, 2010; Fernandes Martins et al., 2010) although they are also possibly fed by groundwater (Chaves, 1999). The wet period usually lasts from November to March, and the dry period lasts from March to November (Table 3.1 and Figure 3.2), although there are both inter- and intra-annual variations.

From the NW 4, situated close to Giara di Gesturi (39°45’ N 8°58’ E), eleven temporary ponds included under a Special Area of Conservation for the Natura 2000 Network (ITB041112) were studied. They are located at 600 m a.s.l. on a steep-sided basaltic plateau of 42 km^2 in southern Sardinia island on hydromorphic soils, with a clay texture and slow drainage. The area is included within the ‘Csa’ Köppen-Geiger climate classification, which means that the climate is warm temperate, with hot and dry summers (Kottek et al., 2006). There is a seasonal distribution of the rainfall, which is at a minimum in the summer and a maximum in the autumn. Snowfall on the plateau is not rare. All the ponds are filled by rainwater and are temporary with a hydroperiod that usually lasts from October to June. The hydroperiod is followed by a dry period from June to October (Table 3.1 and Figure 3.2).

Table 3.1. Description of the mean, mean maximum and minimum annual temperature and mean annual total precipitation of the 4 study sites from this thesis. The 10-year period considered to calculate these data was 2003-2012 for Albera, Guils de Cerdanya and Giara di Gesturi; and 2004-2013 for Vila Nova de Milfontes. Input data: NASA Langley Research Center (LaRC) POWER Project funded through the NASA Earth Science/Applied Science Program.

Study site	Mean annual temperature (°C)	Mean maximum annual temperature (°C)	Mean minimum annual temperature (°C)	Mean annual total precipitation (mm)
Albera (NW 1)	15.79	23.84	8.83	604.717
Guils de Cerdanya (NW 2)	9.08	19.16	0.50	516.453
Vila Nova de Milfontes (NW 3)	16.78	23.24	10.92	489.658
Giara di Gesturi (NW 4)	16.42	25.95	7.96	480.503

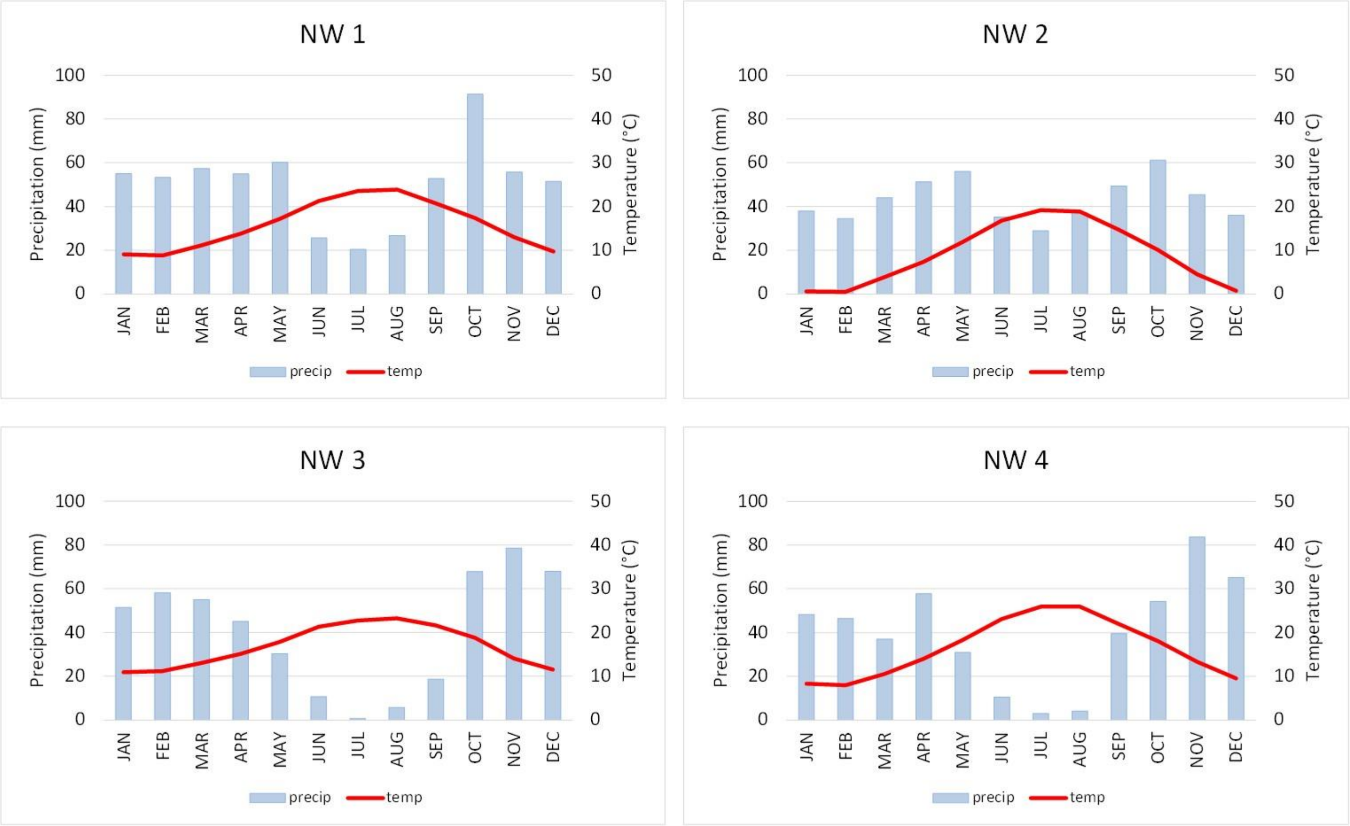

Figure 3.2. Ombrothermic diagrams from the 4 study sites included in this thesis. Input data: NASA Langley Research Center (LaRC) POWER Project funded through the NASA Earth Science/Applied Science Program. Abbreviations are precip (precipitation) and temp (temperature).

3.2. Sampling

Each network was visited once coinciding with the middle phase of the hydroperiod, to avoid the periods when drastic changes in the community structure happen (Boix et al., 2016). Hence, sampling period spanned from February 2012 to April 2013. NW 1 was sampled in February 2012, NW 2 in June 2012, NW 3 in April 2013 and NW 4 in April 2012. During the visit, that usually took us a week, we sampled between 10-12 temporary ponds encompassing the widest range of sizes in each network.

3.2.1 Environmental parameters and pond size

Water temperature (T), dissolved oxygen, conductivity, pH and water-column depth were measured *in situ* during the sampling campaigns. Filtered water samples (250 mL) and unfiltered water samples (250 mL) were collected in each pond and frozen immediately. The dissolved inorganic nutrients (ammonia, nitrite, nitrate, phosphate) were measured from the filtered water samples with the ion chromatography system DIONEX ICS-5000. Dissolved Inorganic Nitrogen (DIN) was then calculated as the sum of the concentrations of ammonia, nitrite and nitrate. Total Inorganic Carbon (TIC), Dissolved Inorganic Carbon (DIC), Dissolved Organic Carbon (DOC), and Total Organic Carbon (TOC) were analysed using the TOC analyser Shimadzu TOC-V CSH and following UNE-EN 1484: 1998 guidelines. Total nutrients [total nitrogen (TN) and phosphorus (TP)] were analysed from unfiltered water samples, following Grasshoff (1983). A nutrient limitation indicator was assessed using the ratio between DIN and TP (molar DIN/molar TP; Ptacnik et al., 2010). Planktonic chlorophyll-a (Chla) content was extracted using 90% acetone, after filtering water samples (Whatman GF/F filters). Chlorophyll-a analyses were carried out with a high-pressure liquid chromatography (HPLC; Waters Pump 1500 Series with an autosampler injector (Waters 717 Plus) and a diode-array detector (Waters PDA 2996) using an adaptation of the method of Zapata et al. (2000), with a C8 reverse phase column and a pyridine mobile phase). To determine the fulvic acids content, a modification of the method described by Hautala et al. (2000) was used: 1) the samples were acidified to $pH < 2.5$ with 1N HCl; 2) twenty-four hours after the acidification, the samples were filtered through a Whatman GF/C filter to eliminate the precipitates of humic acids; 3) the fulvic acids concentration was obtained through spectrophotometry at 350 nm using a UV-1600PC spectrometer (Model VVVR) and applying the regression described in Gan et al. (2007). The macrophyte biomass (g dry weight/m^2) was estimated as the mean dry weight of three replicates of 50.26 cm^2 that were taken randomly from each pond. The dry weight was obtained after oven-drying the material at 60°C over 48 hours. We used a rapid assessment method originally developed for Mediterranean shallow lentic ecosystems (ECELS index; Sala et al., 2004) to evaluate habitat condition, with values ranging from 0 (low habitat quality) to 100 (high habitat quality). This index integrates information on basin littoral morphology, human activity, water descriptors such as odour and transparency, emergent vegetation and hydrophytic vegetation to evaluate habitat condition. The index establishes 5 categories of conservation status (bad, poor, moderate, good and high) according to its numerical score. The maximum surface of the different ponds was estimated using the Google Maps Area Calculator Tool (Daftologic, 2015) and then checked in the field. For more details on the environmental parameters of the study sites see SI Table 4.3.5 and Ballón et al. (2016).

3.2.2 Macrofauna sampling and processing

The macrofauna samples were taken using a dip net with a diameter of 22 cm and a mesh size of 250 µm. The sampling procedure was based on 20 dip-net sweeps in rapid sequence that spanned all of the different mesohabitats. The implemented sampling procedure attempted to solve two problems that we have to face when sampling ponds of different sizes. Because usually bigger habitats require of a greater sampling effort to reflect its biodiversity, some authors have applied a different sampling effort depending on the pond size (e.g., Oertli et al., 2002; Søndergaard et al., 2005). However, in doing this, another problem arises, since if a greater sampling effort is applied, the comparison of the samples is not reliable because, as some authors have indicated (Gaston & Spicer, 2004; Azovsky, 2010), the measures of the community structure change with sampling effort. However, if the same sampling effort is applied in all the ponds, the samples taken from the smallest ponds will reflect the spatial heterogeneity better than those from the largest ponds. Thus, to solve this problem with the trade-off between "reliability" and "sampling effort standardization", we sampled the ponds proportionally according to their size, and then, after homogenizing, we standardized the effort. The procedure was as follows: in small ponds (< 5000 m^2), the sample was obtained by means of 20 dip-net sweeps. In medium ponds (5000 - 20 000 m^2), 40 dip-net sweeps were conducted; then, the capture was homogenized, and only half of this (equivalent to 20 dip-net sweeps) constituted the sample. Finally, in big ponds (> 20 000 m^2), 60 dip-net sweeps were performed; the capture was then homogenized, and one third (equivalent to 20 dip-net sweeps) of the total capture constituted the sample, with the rest being released into the water. The samples were preserved *in situ* in 96% ethanol. Subsequently, in the laboratory, the preservative of the samples was removed, and the individuals were sorted, counted and identified to the species level whenever possible.

Since the sample from each pond contained many individuals, the individuals with greatest size were separated and kept. The rest of the sample was divided into subsamples (Motodo, 1959; Van Guelpen et al., 1982) obtaining several fractions: 1/16, 1/32 and two of 1/64. Then, the individuals with greatest size were identified and counted and posteriorly, starting from the subsample 1/64 (the smallest), the individuals were counted and identified. If the number of individuals counted was below 300, the other subsample of 1/64 was also fully counted and so on until reaching at least 300 individuals. The main identification key used was Tachet et al. (2000), but additional keys were also used. In some cases, specific papers were consulted. The most used keys are listed below.

General keys:

Nilsson, A. (ed.). 1996. Aquatic Insects of North Europe. A taxonomic Handbook (Vols. 1-2). Apollo books, Stenstrup. 714 pp.

Ruffo S. (coord.). 1977-1985. Guide per il riconoscimento delle specie animali delle acque interne italiane. CNR, Collana del Progetto Finalizzato "Promozione della qualità dell'ambiente", AQ/1/233.

Tachet, H., Richoux, P., Bournaud, M., Usseglio-Polatera, P. 2000. Invertebrés d'eau douce: systématique, biologie, écologie. CNRS Editions. Paris, 588 pp.

Amphibians:

Miaud, C. & Muratet, J. 2004. Identifier les oeufs et les larves des amphibiens de France. 202 pp.

Nöllert A. & Nöllert, C. 1995. Los anfibios de Europa. Identificación, amenazas, protección. Omega (eds.). 400 pp.

Diptera:

Sinegre, G., Rioux, J.A. & Salgado, J. 1979. Fascicule de determination des principales especes de moustiques du litoral méditerranéen français. Entente Interdépartementale pour la Démoustication du littoral méditerranéen.

Smith, K.G.V. 1989. An introduction to the immature stages of British flies. Diptera larvae, with notes on eggs, puparia and pupae. In: *Handbooks for the Identification of British Insects* 10/14. Dolling, W.R. & Askew, R.R. (eds.). Royal Entomological Society of London, London. 280 pp.

Chironomidae (larvae):

Wiederholm, T. (ed.). 1983. Chironomidae of the Holarctic Region: Keys and Diagnoses, Part 1: Larvae. Entomologica Scandinavica, supl. 19: 1-457.

Chironomidae (pupae):

Langton, P.H. 1991. A key to pupal exuviae of West Palaearctic Chironomidae. P. H. Langton, Huntingdon (private publ.) 386 pp.

Culicidae:

Cranston, P. S., Ramsdale, C. D, Snow, K. R, White, GB. 1987. Adults, Larvae and Pupae of the British Mosquitoes (Culicidae): A Key. Freshwater Biological Association, Ambleside. 152 pp.

Encinas Grandes, A. 1982. Taxonomía y biología de los mosquitos del área Salmantina. Diptera (Culicidae). CSIC, Centro de Edafología y Biología Aplicada, Ed. Universidad de Salamanca. 437 pp.

Coleoptera:

Angus, R. 1992. Insecta Coleoptera: Hydrophilidae: Helophorinae. In: Schwoerbel, J. & Zwick, T. (eds.). Süßwasserfauna von Mitteleuropa. Band 20/10-2. Gustav Fischer Verlag, Stuttgart. 142 pp.

Franciscolo, M.E. 1979. Coleoptera. Haliplidae, Hygrobidae, Gyrinidae, Dytiscidae. In: *Fauna d'Italia.* Baccetti, B. et al. (eds.). Edizioni Calderini, Bologna. 804 pp.

Hemiptera:

Jansson, A. 1986. The Corixidae (Heteroptera) of Europe and some adjacent regions. Acta Entomologica Fennica 47: 1-94.

Nieser, N., Baena, M., Martínez-Avilés, J. & Millán, A. 1994. Claves para la identificación de los heterópteros acuáticos (Nepomorpha y Gerromorpha) de la Península Ibérica - Con notas sobre las especies de las Islas Azores, Baleares, Canarias y Madeira. 112 pp.

Odonata:

Askew, R.R. 1988. The dragonflies of Europe. Harley books, Colchester. 291 pp.

Heidemann, H. & Seidenbusch, R. 2002. Larves et exuvies des libellules de France et d'Allemagne. Societé française d'Odonatologie, Bois d'Arcy, France. 416 pp.

Trichoptera:

Wallace, I.D. 1990. Key to the Case-bearing Caddis Larvae of Britain and Ireland. Freshwater Biological Association. 240 pp.

Hydrachnidia:

Di Sabatino, A., Gerecke, R., Gledhill, T. & Smit, H. 2010. Acari: Hydrachnidia II. In: Gerecke, R. (ed.), *Chelicerata: Acari II*. Süßwasserfauna von Mitteleuropa, Vol. 7, 2–2, Elsevier Spektrum Akademischer Verlag, Heidelberg. 234 pp.

4. RESULTS

CHAPTER 1

Pond size effect on macrofauna community structure in a highly connected pond network

OVERVIEW

The biogeographical principle that larger areas contain more species than smaller areas has more often been assumed than tested. In this sense, contradictory results have been published in studies on the relationship between water body size and species richness in temporary waters. Pond size can have an effect on the structure and composition of the macrofauna community, but this effect can be modified by other environmental factors such as water trophic state, habitat structure and spatial connectivity within the ecosystem. We sampled the aquatic macrofauna (from midges to amphibians) from a network of twelve Mediterranean temporary ponds in southwestern Portugal with a strong size gradient (245 – 78 652 m^2), also taking into account three environmental factors that can modulate the relationship between pond size and community structure: connectivity, water trophic state and habitat structure. Our aim was to test the importance of pond size in macrofaunal structure and composition. Pond size was not related to any of the three environmental factors included in this study. Our results noted an unclear relationship between pond size and macrofauna, since we found a significant effect on community composition but did not find an effect on community structure parameters such as richness, taxonomic diversity or body size diversity. The high connectivity among ponds seems to be a plausible explanation for the observed pattern.

BACKGROUND

One of the principles of the "island biogeography theory" is that a relationship exists between the size of an island and its species richness. Moreover, this theory assumes that islands that are close to one another will present higher immigration rates than islands that are further apart, and closer islands will thus have more species in common than islands that are further apart (MacArthur & Wilson, 1967). Aquatic environments have often been considered as islands in the varied literature on the species-area relationship, and this relationship has been observed in a high range of organisms, from macro- to microorganisms (e.g., King *et al.* 1996; Oertli *et al.*, 2002; Reche *et al.*, 2005). This rule offers attractive applications for conservation biology, but the relationship between this principle and nature conservation has been more assumed than tested (Oertli *et al.*, 2002). In this sense, some studies performed in permanent ponds (Friday, 1987; Gee *et al.*, 1997) have not found a significant relationship between pond area and macroinvertebrate community richness. In the case of temporary ponds, contradictory results exist, since a relationship between pond size and species richness has been reported in some studies (e.g., Ebert & Balko, 1984; March & Bass, 1995; King *et al.*, 1996; Spencer *et al.*, 1999), but works failing to observe this relationship have also been published (Bilton *et al.*, 2001). Moreover, it is also remarkable that most of the published results from ponds, whether permanent or temporary (Brönmark, 1985; King *et al.*, 1996), have been based only on one or two biotic groups, but there are few studies in which different taxonomic groups are compared at the same time (but see Oertli *et al.*, 2002). In this regard, pond size has previously been identified as a determinant factor for species richness in many invertebrate groups, but its influence on the entire macroinvertebrate community is less well documented (Gee *et al.*, 1997). The influence of habitat size has been analysed not only in terms of species richness but also other ecological parameters such as the food chain length, the proportion of predators and trophic interactions (Spencer *et al.*, 1999; McCann *et al.*, 2005; Arim *et al.*, 2010).

Therefore, the pond size itself can have an effect on the macrofauna community, but this effect can be modulated by other environmental factors such as the water trophic state, the habitat structure and connectivity. First, many researchers have emphasised the importance of the water chemistry or trophic conditions of ponds in determining the structure of freshwater macroinvertebrate assemblages (e.g., Friday, 1987; Jeffries, 1991; Heino, 2000). It is known that an increase in nutrients (as a proxy of trophic state) can lead to a decline in species richness in ponds (e.g., Jeppesen *et al.*, 2000; Declerck *et al.*, 2005; Boix *et al.*, 2007). Changes in nutrient loading result in changes in community structure (Jeppesen *et al.,* 2000). Moreover, diversity indices, and particularly species richness, are sensitive to some ecological stress factors such as eutrophication (Jeppesen *et al.,* 2000; Declerck *et al*., 2005). However, most studies on species richness and diversity have focused only on one or two groups of taxa, and few of them have compared the response of various trophic levels/groups of taxa to changes in the trophic state (but see Declerck *et al*., 2005). Second, the role of macrophytes as physical structures that increase habitat complexity or heterogeneity in aquatic ecosystems is widely recognised. Macrophytes affect animal assemblages and promote biodiversity through a chain of mechanisms related to habitat complexity (Thomaz & Cunha, 2010). In this sense, many studies have noted that macrophytes can influence the distributions of aquatic invertebrates by affecting food availability (Campeau *et al*., 1994) and predation (Schriver *et al*., 1995; but see Gascón *et al*., 2013) because plants provide refuge (Jeppesen *et al*., 1997). Finally, the success of reaching a suitable habitat depends on the explicit spatial configuration, the connectivity of different habitat types and the surrounding landscape. Thus, the dispersal of individuals among habitats contributes to changes in community structure (Michels *et al*., 2001; Van de Meutter *et al*., 2007). In general, water bodies that are more connected can be accessed by a greater number of species than those that are more isolated (Olden *et al*., 2001). For instance, in the case of amphibians, both landscape and pond connectivity are very important for the long term persistence of their populations because they may buffer the stochastic events that frequently occur in Mediterranean ponds (Ribeiro *et al*., 2011). In the case of macroinvertebrates, the connectivity between ponds can increase the similarities between communities compared to non-connected or indirectly connected ponds (Van de Meutter *et al*., 2007).

In the present study, we test the effect of pond size on the macrofaunal community in temporary ponds. To do this, we sampled the macrofaunal assemblages of Mediterranean temporary ponds located in a network with a pronounced pond size gradient. Factors that potentially modulate the effects of pond size on the aquatic community (water trophic state, habitat structure and connectivity) were also assessed and included in our analyses. However, because we sampled a pronounced size gradient, we expect to find a strong size effect on the structure and composition of the macrofauna. To test this, we first studied the relationships between each of the three environmental factors (water trophic state, habitat structure and connectivity) and pond size, and we then established the relationships between community structure parameters and composition and pond characteristics.

METHODOLOGICAL APPROACH

Study site

This study was carried out in the twelve temporary ponds situated in NW 3. These twelve ponds were intentionally chosen to include the broadest possible range of sizes available in the study area (245 – 78 652 m^2) (Figure 4.1.1). More information on the study site is provided in the 'General methodology' section.

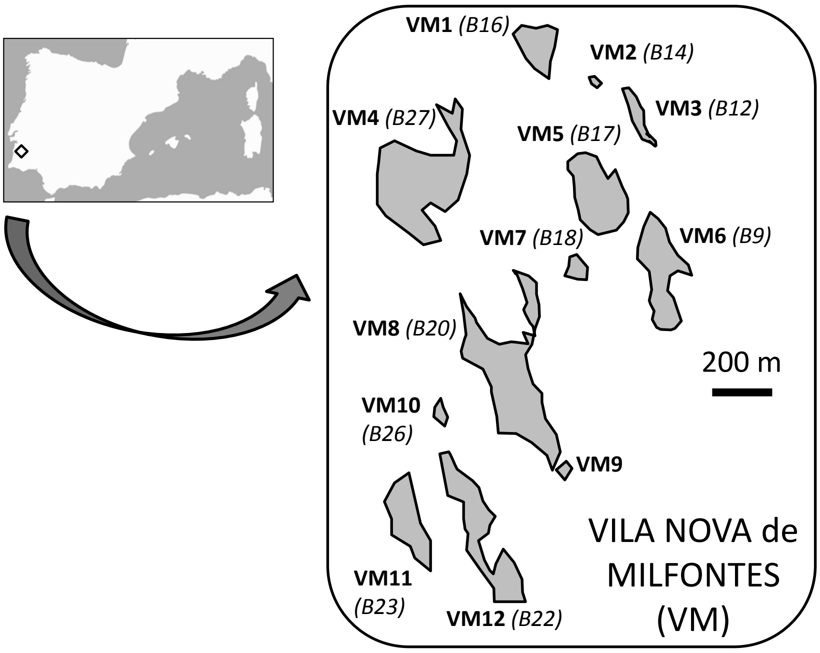

Figure 4.1.1. Study site locations showing pond positions within the network. The original codification of the ponds used by Caramujo & Boavida (2010) and Chaves (1999) is shown in brackets.

Sampling and processing

To sample the macroinvertebrates and to obtain the water parameters from each pond we followed the procedures described in the 'General methodology' section. In this study, individuals were identified to species level whenever possible, except in the case of chironomids, which were identified to subfamily. At least 26 individuals for each taxon and sample were randomly chosen for measurements to estimate individual biomass.

Connectivity, habitat structure and water trophic state determination

The spatial connectivity between ponds was measured using the equation in Henriques-Silva *et al.* (2013): Average Connectivity= $\frac{1}{n}\sum_{\substack{i=1\\ i\neq j}}^{n} p_{jk}\exp(-d_{ij})$

where "Average Connectivity" measures the average geographic distance (based on the latitude/longitude) across pond *i* for the *k*th species across all other *n*-1 ponds, and *p* indicates the presence (1) or absence (0) of the *k*th species in the *j*th pond. In cases where species *i* was found only in one pond, we assigned for that species the maximum distance between two sampled ponds as its connectivity value (i.e., the smallest connectivity). For each pond, the overall connectivity was calculated as the average connectivity value for all species present in it.

We used the macrophyte biomass (Macrophytes_DW) per pond as a proxy for the habitat structure of each pond (e.g., Thomaz & Cunha, 2010). The macrophyte biomass (g DW/m^2) was estimated as the mean dry weight of three replicates of 50.26 cm^2 that were taken randomly from each pond. The dry weight was obtained after oven-drying the material at 60°C over 48 hours. Finally, to determine the trophic state of the system, TRIX (the trophic index) was calculated for each pond. This index is based on the planktonic chlorophyll *a*, oxygen saturation, total nitrogen and phosphorus. Numerically, the index is scaled from 0 to 10, covering a wide range of trophic conditions from oligotrophy (0) to eutrophy (10) (Vollenweider *et al*., 1998). It was calculated using the following equation:

$$\text{TROPHIC INDEX}=(\log[Ch*aD\%O*N*P]-[-1.5])/1.2$$

where *Ch* is the chlorophyll *a* (mg/m^3), *aD%O* is the oxygen as its absolute deviation (in percentage) from saturation (abs |100-%O|), *N* is the total nitrogen (mg/m^3) and *P* is the total phosphorus (mg/m^3).

Community structure parameters

The following ecological parameters were calculated: (i) the number of taxa per sample, i.e., taxa richness (*S*); (ii) the taxa diversity assessed by means of the Shannon-Wiener index (*H'*), which is based on the numerical abundance of each identified taxon; and (iii) the IFO (index of faunal originality) as a metric to evaluate the rarity of the species in each sample. The IFO was calculated according to Puchalski (1987):

$$\text{IFO}=\frac{\sum\left(\frac{1}{M_i}\right)}{S}$$

where *M* is the total number of samples in which species *i* occurs (from *i* = 1 to *S*), and *S* is the number of species in the corresponding sample. One way to analyse the taxonomic relatedness among the organisms in a sample is by calculating different phylogenetic or taxonomic metrics. These metrics were proposed as useful tools to measure some biodiversity aspects that were helpful as conservation criteria (e.g., Polasky *et al.*, 2001; Barker, 2002). We calculated taxonomic relatedness based on (i) the taxonomic distinctness (TD), (ii) the average taxonomic distinctness (ATD) and (iii) the variation in taxonomic distinctness (VTD) using PRIMER-E v.6 (Clarke & Gorley, 2006). The first index, TD, is the average path length between any two randomly chosen individuals, conditional on them being from different species (Clarke & Warwick, 1998). The second index, ATD, is the mean path length through the taxonomic tree connecting every pair of species (Clarke & Warwick, 2001). Finally, the VTD is simply the variance of these pairwise path lengths and reflects the unevenness of the taxonomic tree. It can be used to compare samples with similar ATD but different taxonomic tree structure.

Faunal biomass estimates (such as dry weight) were obtained from the allometric relationship between an individual's weight and the length of its body (Meyer, 1989; Smit *et al.*, 1993; Arias & Drake, 1994; Benke *et al.*, 1999; Boix, 2000; Baumgärtner & Rothhaupt, 2003). The body size diversity (μ) was calculated for each sample. It is used to describe the shape of the biomass size spectra, and its use to study community structuring seems advantageous over the traditional taxonomic approach, since body size can be more directly related to metabolism and energy transfer within communities (Woodward *et al.*, 2005). To calculate the body size diversity, we used the non-parametric estimation proposed by Quintana *et al.* (2008). This measure takes the form of an integral involving the probability density function of the body size of the individuals described by the following equation:

$$\mu = -\int_0^{+\infty} p_x(x) \log_2 p_x(x) dx$$

where $p_x(x)$ is the probability density function for size. Non-parametric kernel estimation was used as a probability density function after the data were standardised by dividing the sample data by their geometric mean value (Quintana *et al.*, 2008). The body size diversity was obtained using the software Diversity08. The body size geometric mean was obtained from the same software and provided information about the mean body size of the organisms observed in each sample. Finally, the slope of the normalised biomass size spectrum was obtained for each sample.

Statistical analyses

A principal component analysis (PCA) was performed to determine the characteristics that best explained the variability in the normalised physical and chemical dataset. Non-parametric multi-dimensional scaling (NMDS) was performed to visualise the similarities in pond composition using the abundance data and the Bray-Curtis similarity coefficient. The abundance data were previously standardised by dividing, in each case, the sample values by the total abundance per sample. The vectors of the environmental variables (connectivity, TRIX, macrophyte biomass) and pond sizes were then fitted into the ordination space (NMDS) to detect possible associations between the patterns of species composition and environmental variables using the 'envfit' function of the 'vegan' package in R (R Core Team, 2015), and statistical significance was evaluated by 999 random permutations.

To analyse if the community structure parameters responded to pond size, simple linear regression models were performed. Previously, we had tested the assumptions of normality and homoscedasticity using the R function 'mcheck'. Pond size was the explanatory variable, and the different community structure parameters (previously calculated) were used as the dependent variable in each model. Moreover, to test the possible influence of the other environmental factors that can modulate the pond size effect, we also used simple linear regression models, but this time taking each of the environmental variables (connectivity, TRIX and macrophyte biomass) as the explanatory variable in each case. Finally, to identify whether the selected environmental variables (connectivity, TRIX and macrophyte biomass) were affected by changes in pond size (explanatory variable), consequently covariation would exist among them, we used different simple linear regression models. The PCA was carried out with PRIMER v.6. The rest of the data analyses were performed in R ver.3.1.2 using the package 'car'.

OUTCOMES

The macrofauna found in the entire pond network included 78 taxa, most of which were insects (59 taxa). The best represented orders of insects were Coleoptera (29 taxa), Diptera (12 taxa) and Heteroptera (11 taxa) (for more details on the fauna, see Appendix 1). When analysing the taxa richness of each pond for the major taxonomic groups, we detected that the amphibian and dipteran richness was almost the same among the ponds. In contrast, the richness of heteropterans, odonates and coleopterans was more variable. The greatest coleopteran richness was found in the ponds of intermediate size. Pond VM6, with an intermediate size, presented the greatest richness both in heteropterans and odonates (Figure 4.1.2).

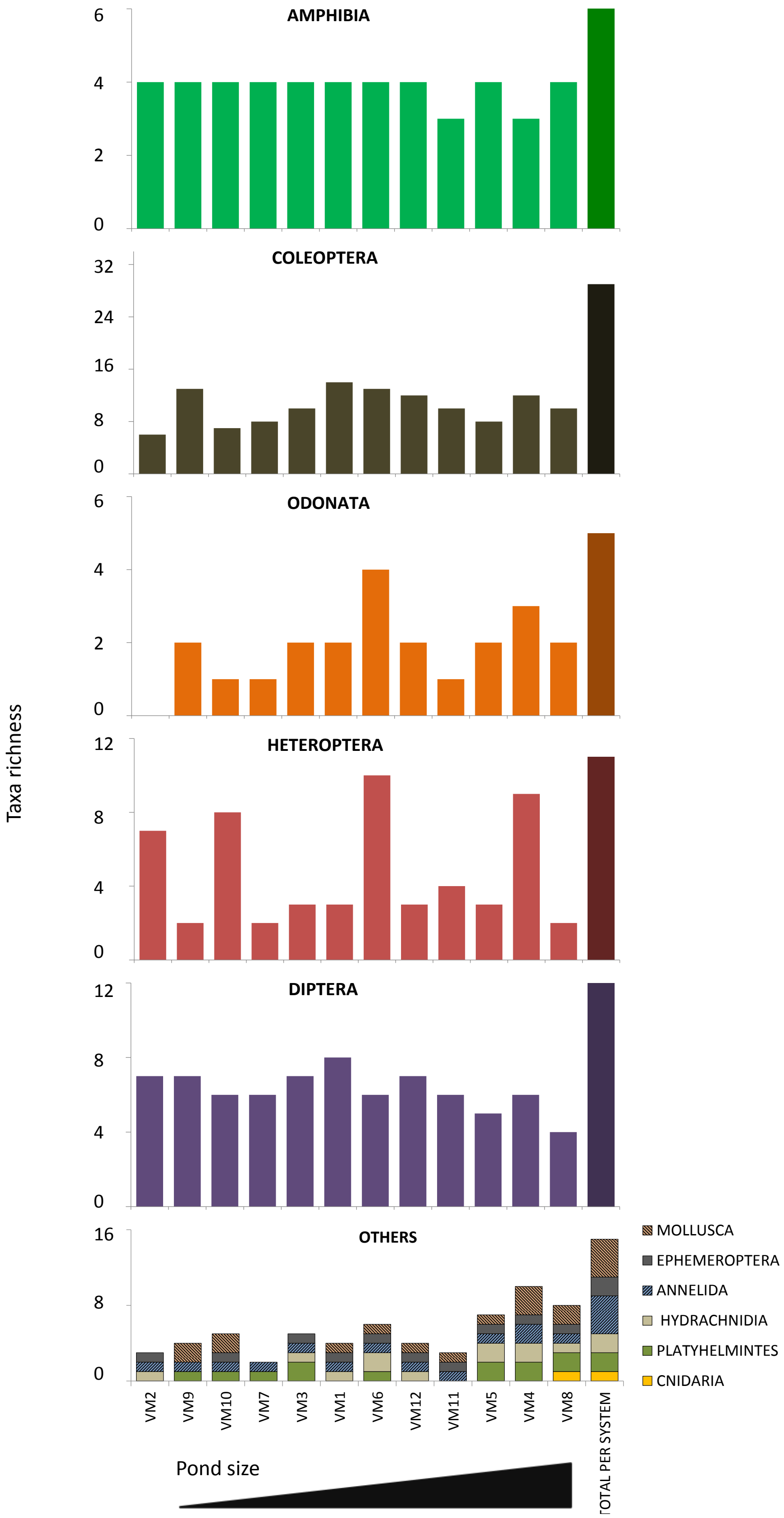

Figure 4.1.2. Taxa richness for each major groups of organisms in each pond. Right bar indicates the total taxa richness per system. Pond size increases from left to right.

The first two axes of the PCA explained 52.3 % of the variance. PC1 explained 25.3 % of the variance, while PC2 explained 20.2 % (Figure 4.1.3). The variables with the highest contribution to PC1 were the TN-N, mean temperature (T), maximum depth (Depth) and conductivity (Cond). pH, DIC, TIC and DIN were the main variables contributing to PC2. Ponds VM11 and VM12 had the most distinct physical and chemical characteristics in relation to the others, as shown in the PCA plot (Figure 4.1.3). Moreover, VM12 had the highest trophic conditions (TRIX index = 7.3; Table 4.1.1). In general, the system can be considered to be limited by N, with low values of pH, dissolved oxygen and DIN. However, it had high values of phosphates, TN, TOC, fulvic acids and macrophyte biomass.

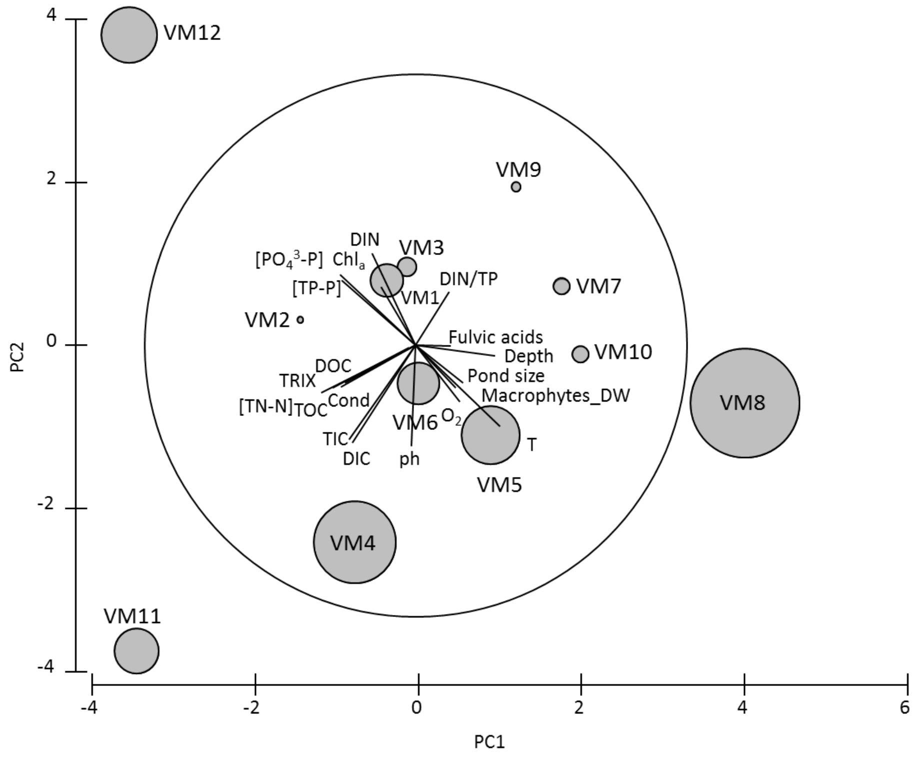

Figure 4.1.3. PCA plot showing ponds position in relation to the physical and chemical characteristics of the water. The size of the circles is proportional to pond size. The closer a variable is to the circle of correlations, the better it can be reconstructed from the first two components (and the more important it is to interpret these components); the closer to the center of the plot a variable is, the less important it is for the first two components. The codes correspond to the sampled ponds (see Figure 4.1.1).

Table 4.1.1. Mean and range of variation of the physical and chemical characteristics of the ponds studied.

Water characteristics	Mean (Range)
Maximum depth (cm)	62.33 (31-106)
Temperature (°C)	21.53 (17.40-23.90)
Conductivity (µS/cm)	746.53 (390-1274.67)
pH	6.43 (5.36-7.28)
Dissolved oxygen (mg/L)	4.58 (2.82-6.61)
Ammonium (mg NH_4^+-N/L)	0.02 (0.001-0.062)
Nitrate (mg NO_3^--N/L)	0.003 (0.002-0.009)
Nitrite (mg NO_2^--N/L)	0.004 (0.003-0.006)
Phosphate (mg PO_4^{3-}-P/L)	0.02 (0.004-0.096)
Total nitrogen (mg NT-N/L)	2.77 (1.87-3.80)
Total phosphorus (mg PT-P/L)	0.13 (0.03-0.64)
Chlorophyll *a* (µg/L)	10.47 (0.27-40.87)
Trophic state index	4.96 (3.89-7.27)
DIC (mg C/L)	12.96 (2.06-73.64)
TIC (mg C/L)	14.30 (3.57-75.19)
DOC (mg C/L)	54.82 (40.14-79.44)
TOC (mg C/L)	57.67 (40.14-80.12)
molar DIN/molar TP	0.67 (0.10-2.27)
Macrophyte biomass (g DW/cm^2)	0.19 (0.12-0.31)
% fulvic acids	65.04 (50.89-86.49)

In contrast, when looking at the relationships between the pond community composition similarities (NMDS) and the environmental factors analysed, pond size was a unique variable, showing a significant relationship ($p = 0.006$; Fig. 4.1.4A). The abundance of some taxa, such as *Gyraulus laevis* (Planorbidae), *Piona* sp. (Pionidae) and *Aeshna mixta* (Aeshnidae), increased in larger ponds, whereas the abundance of other taxa, such as *Agabus* sp. (Dytiscidae), *Chaoborus flavicans* (Chaoboridae) and *Culex theileri* (Culicidae), had their maximum density in smaller ones (Figure 4.1.4B). The rest of the variables (connectivity, TRIX, macrophyte biomass) did not show any relationships with the community composition data.

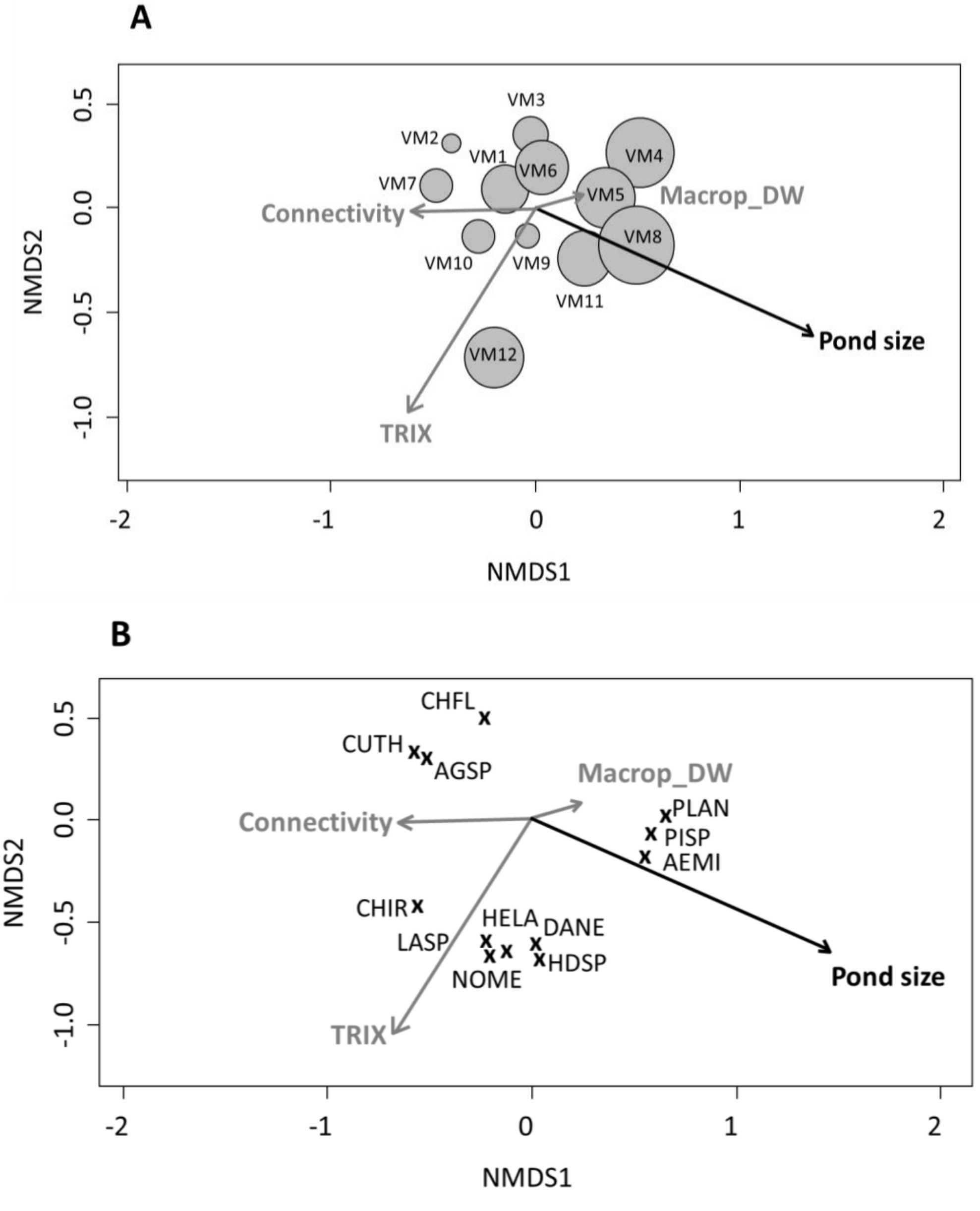

Figure 4.1.4. **A**) NMDS plot showing ponds identified by means of their code (see Figure 4.1.1) according to their taxonomic composition. The size of the circles is proportional to pond size. Environmental factors (connectivity, TRIX, macrophytes biomass (Macrop_DW), and pond size (as the natural logarithm of pond size) are represented by arrows. The black arrow indicates the environmental variable with a significant effect (*p*=0.006). Grey arrows represent non-significant variables. **B**) NMDS plot showing the taxa with a significant effect (*p*≤0.05) on the community composition. Acronyms stand for: PLAN (*Gyraulus laevis*), PISP (*Piona* sp.), AEMI (*Aeshna mixta*), DANE (*Dicranomyia/Atypopthalmus/Neolimonia*), HDSP (*Hyphydrus* sp.), HELA (*Helophorus lapponicus*), NOME (*Notonecta meridionalis*), LASP (*Laccophilus* sp.), CHIR (Chironominae), AGSP (*Agabus* sp.), CUTH (*Culex theileri*) and CHFL (*Chaoborus flavicans*).

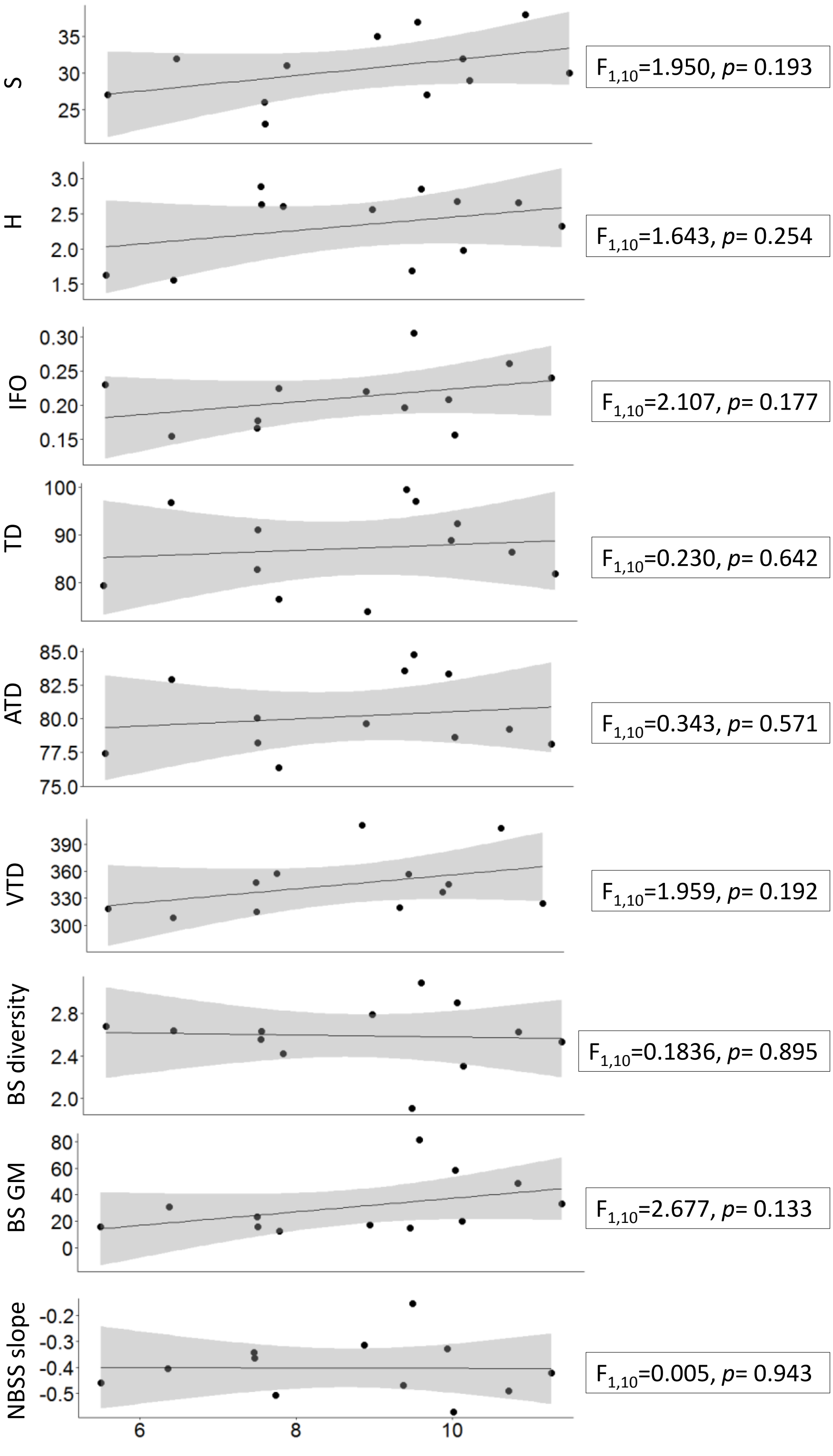

Figure 4.1.5. Relationship between pond size (natural logarithm of pond size) and the different community structure parameters. The results obtained by means of linear regression models are shown; the fitness of the regression coefficients was not statistically different from the intercept-only model (*p*> 0.05). Acronyms and symbols stand for: S (richness), *H'* (Shannon-Wiener index), IFO (Index of Faunal Originality), TD (Taxonomic Distinctness), ATD (Average Taxonomic Distinctness), VTD (Variation in Taxonomic Distinctness), BS diversity (body size diversity) BS GM (body size geometric mean), NBSS slope (normalised biomass-size spectra slope).

In looking at the community structure parameters (regression results), no significant relationships arose, neither for pond size (Figure 4.1.5) nor for the rest of environmental factors tested (Table 4.1.2). In analysing the relationship between pond size and the environmental variables that can potentially modulate the pond size effect, no significant relationships were found (connectivity: $F_{1,10} = 3.3460$, $p = 0.097$; TRIX: $F_{1,10} = 0.0002$, $p = 0.990$ and macrophyte biomass: $F_{1,10} = 0.2383$, $p = 0.636$). Therefore, no covariation exists between pond size and the environmental variables tested.

Table 4.1.2. The statistics F and *p* are shown for the different linear regression models performed between community structure parameters (response variables) and environmental variables (explanatory variables). The fitness of the regression coefficients was not statistically different from the intercept-only model ($p > 0.05$). See Figure 4.1.5 for acronyms.

	Connectivity	TRIX	Macrophytes biomass
S	$F_{1,10}=0.001$; $p= 0.9817$	$F_{1,10}=0.003$; $p=0.955$	$F_{1,10}=0.690$; $p=0.426$
H'	$F_{1,10}=0.036$; $p=0.853$	$F_{1,10}<0.001$; $p=0.991$	$F_{1,10}=0.598$; $p=0.457$
IFO	$F_{1,10}=0.548$; $p=0.476$	$F_{1,10}=0.181$; $p=0.679$	$F_{1,10}=2.314$; $p=0.159$
TD	$F_{1,10}= 3.455$; $p= 0.0927$	$F_{1,10}=1.544$; $p=0.242$	$F_{1,10}=0.167$; $p=0.691$
ATD	$F_{1,10}= 0.875$; $p=0.372$	$F_{1,10}=2.963$; $p=0.116$	$F_{1,10}=0.390$; $p=0.546$
VTD	$F_{1,10}=0.437$; $p=0.524$	$F_{1,10}=0.847$; $p=0.379$	$F_{1,10}=0.273$; $p=0.613$
BS diversity	$F_{1,10}=1.217$; $p=0.296$	$F_{1,10}=0.254$; $p=0.625$	$F_{1,10}=0.157$; $p=0.700$
BS GM	$F_{1,10}=0.155$; $p=0.702$	$F_{1,10}=0.710$; $p=0.419$	$F_{1,10}=0.101$; $p=0.757$
NBSS slope	$F_{1,10}=0.166$; $p=0.693$	$F_{1,10}=0.465$; $p=0.511$	$F_{1,10}=0.459$; $p=0.513$

DISCUSSION

Size has been noted on several occasions as being a determinant factor for the community composition of ponds (Rundle *et al.*, 2002; Vanschoenwinkel *et al.*, 2009). Similarly, in the case of lakes, several studies have shown an influence of the ecosystem size on community structure (e.g., Post *et al.*, 2000; Søndergaard *et al.*, 2005). Nevertheless, when we focus on ecosystems with higher environmental variability, such as Mediterranean temporary ponds, the influence of ecosystem size has been less documented. In our study, we did not find a strong effect of pond size on macrofauna, since size seems to influence the faunal composition but not structural parameters. Similarly, Rundle *et al.* (2002) found that the abundance of four coleopteran species was positively related to pond size. Thus, they found variation in the invertebrate composition, which also occurred in our study. Considering our results, none of the community structure parameters had a significant relationship with pond size. Likewise, Gascón *et al.* (2009) studied the relationships between six parameters that we also tested and the size of some temporary ponds, and they also found no significant relationships. Although Jeppesen *et al.* (2000) found an increase in the Shannon-Wiener diversity index for phytoplankton with lake area, we did not find a significant relationship between pond size and the Shannon-Wiener diversity index for macrofauna. Nevertheless, this lack of relationship is not rare, since some studies have found a significant positive relationship between habitat area (lakes or seas) and species richness (Søndergaard *et al.*, 2005; Azovsky, 2010), but many others were unable to find such relationships (García-Valdecasas *et al.*, 1984; Friday, 1987; Jeffries, 1991). Moreover, Oertli *et al.* (2002), when working on permanent ponds, observed a positive relationship between area and the richness of some macroinvertebrate taxa such as Odonata and Gastropoda, but they found no significant relationships for Sphaeriidae, Coleoptera or Amphibia. In accordance with this, Della Bella *et al.* (2005) noted that the number of Coleoptera was not related to the pond size (including both permanent and temporary), presenting a different pattern in species richness from Odonata, Chironomidae and Hemiptera. In temporary environments, Spencer *et al.*, (1999) found that the species richness and predator-prey ratio were positively correlated with the maximum pool surface area, while Bilton *et al.* (2001) found no pond size effect on the species richness nor on the predator-prey ratio. The latter authors explained these contrasting results by the existence of a critical pond size, above which the detection by colonists is more likely to occur. In fact, we have noticed that the studies that found a positive relationship between pond size and species richness in temporary ponds (Ebert & Balko, 1984; March & Bass, 1995; Spencer *et al.*, 1999) included smaller water bodies than those in studies that did not find this relationship, such as the current study or the study by Bilton *et al.* (2001). Furthermore, some authors (e.g., Oertli *et al.*, 2002; Gaston & Spicer, 2004) have stated that most studies that found a positive relationship between area and richness did not remove the sampling effort effect. This does not seem to be the case in our study, since we removed the sampling effort effect during field sampling. In summary, our results reflect an unclear effect of pond size in temporary habitats because we found a significant relationship between pond size and community composition, but we found no relationship between pond size and any of the community structure parameters.

The effect of pond size on community structure and composition can be modified by other factors such as habitat structure, water chemistry and connectivity. However, the indirect effects of pond size on community structure (i.e., larger ponds have different water physical and chemical characteristics than those that are smaller) seem to be weak in Mediterranean temporary ponds (Ballón *et al*., 2016). Aquatic macrophytes play an important role in habitat structure and are highly influential in the composition of the associated fauna since they increase food availability and therefore attract other organisms, influencing interspecific relationships (Thomaz & Cunha, 2010). On the other hand, because bigger ponds may have larger drainage basins than smaller ponds, they may receive greater amounts of nutrients, and this may lead to a eutrophic state (Wetzel, 2001). However, in our study, the most eutrophic pond (VM12) was not the largest in the study area. As far as we know, only a few studies have attempted to analyse the relationship between pond size and connectivity (e.g., Scheffer *et al*., 2006). However, pond size may affect the probability of species colonisation and extinction, and this probability may also be influenced by the spatial location of the pond (Spencer *et al*., 1999). Moreover, larger ponds are likely to act as greater focus of dispersal than smaller ponds when there is a patchy distribution within the pond network, but the scope of dispersal would not be the same for big isolated ponds. In our study, the connectivity of each pond in relation to the others can be considered high, and it would thus not be a problem for at least some macroinvertebrate species with high dispersal rates to move from one pond to another. Thus, the high spatial connectivity established among the pond network could explain the absence of significant differences in the community structure parameters among the ponds. However, we did not find significant relationships between any of the three environmental factors analysed (water trophic state, habitat structure and connectivity) and pond size. Therefore, the effect of pond size detected in the faunal composition is not due to a covariation effect because larger ponds showed greater connectivity, a higher trophic state or more habitat structure.

Metacommunities (Hanski & Gilpin, 1991; Wilson, 1992; Holyoak *et al*., 2005) constitute a good theoretical framework to improve conservation strategies, allowing the inclusion of regional processes in management. In this sense, the pond network of Vila Nova de Milfontes can be considered to be a metacommunity with high spatial connectivity within the “pondscape” (for information on the pondscape concept, see Baguette *et al*., 2012). Thus, the exchange of a high proportion of species that characterises a metacommunity

(Leibold *et al*., 2004) makes it especially important to focus conservation strategies on the entire pond network and not only on a few ponds to maintain regional biodiversity. The absence of differences, for instance, in species richness and diversity among ponds of different sizes should be taken into account in conservation policy and management, since pond size has sometimes been used as a criterion to prioritise resources for conservation (Oertli *et al*., 2002). Hence, it is important to promote the conservation of ponds regardless of their size. Small ponds can harbour the same species as bigger ponds, and the small ponds can act as stepping stones between larger ponds, helping to maintain metacommunity dynamics and stability (Leibold *et al*., 2004; Vanschoenwinkel *et al*., 2009). Thus, as has also been previously demonstrated, it is important to preserve pond networks because the spatial distribution of ponds influences pond quality as well as species distributions and dynamics (Gibbs, 2000; Jeffries, 2005).

CHAPTER 2

Dispersal mode and spatial extent influence distance-decay patterns in pond metacommunities

OVERVIEW

Assuming that dispersal modes or abilities can explain the different responses of organisms to spatial or environmental distances, the distance-decay relationship is a useful tool to evaluate the relative role of local environmental structuring *versus* regional control in community composition. Based on continuing the current theoretical framework on metacommunity dynamics and based on the predictive effect of distance on community similarity, we proposed a new framework that includes the effect of spatial extent. In addition, we tested the validity of our proposal by studying the community similarity among three biotic groups with different dispersal modes (macrofaunal active and passive dispersers and plants) from two pond networks, where one network had a small spatial extent, and the other network had an extent that was 4 times larger. Both pond networks have similar environmental variability. Overall, we found that environmental distance had larger effects than spatial distances in both pond networks. Moreover, our results suggested that species sorting is the main type of metacommunity dynamic shaping all biotic groups when the spatial extent is larger. In contrast, when the spatial extent is smaller, the observed distance-decay patterns suggested that different biotic groups were mainly governed by different metacommunity dynamics. While the distance-decay patterns of active dispersers better fit the trend that was expected when mass effects govern a metacommunity, passive dispersers showed a pattern that was expected when species sorting prevails. Finally, in the case of plants, it is difficult to associate their distance-decay patterns with one type of metacommunity dynamic.

BACKGROUND

Metacommunity ecology describes a group of local communities that interact with each other through dispersal and generate both local and regional interactions that influence local community assemblages (Hanski & Gilpin, 1991; Wilson, 1992; Holyoak et al., 2005). Thus, metacommunity dynamics affect regional biotas, and this effect feeds back into the patterns of local variation (Leibold et al., 2004; Logue et al., 2011). Recently, community ecology has been attempting to elucidate the specific role of regional and local processes in determining metacommunity functioning (Cottenie & De Meester, 2004; Leibold & Norberg, 2004; Moritz et al., 2013). In this sense, assessing the relative importance of environmental control against spatial distances appears to be a crucial aspect to disentangle which type of metacommunity dynamic is acting (Cottenie & De Meester, 2004).

The distance-decay of similarity is a valuable tool to understand species assemblage responses to environmental and spatial variability (Morlon et al., 2008). For instance, a decay in similarity is usually observed when spatial distance between patches increases due to the dispersal limitation encountered when some organisms disperse across the landscape, and hence, widely separated points will harbour different communities (Harte, 2003). Similarly, environmental distance decay might be observed when communities are environmentally controlled (Heino, 2013a). In fact, it is possible that both distances affect metacommunity dynamics and structure, and measuring the distances is an approach to evaluating the relative role of the spatial configuration of patches versus patch environmental variability (Heino, 2011; Astorga et al., 2012). Interestingly, a proposal has been developed (Heino, 2013a) that links the patterns observed in the distance-decay of similarity and the dynamics governing metacommunities. Based on that proposal, we can identify three predictions that differ in terms of the effect of distance on community similarity according to three metacommunity paradigms: 1) species sorting, when a decay in similarity is observed for the environmental distance but not for the spatial distance; 2) neutral model, which is the opposite situation to the previous one, showing a decay in similarity for the spatial distance but not for the environmental distance, and 3) mass effects, showing a significant decay in similarity for both distances. However, Heino’s proposal does not incorporate the effect of the spatial extent on the rate of decay (i.e., the slope) of community similarity, which is usually lower at larger scales (Soininen et al., 2007c).

On the other hand, to fully understand the importance of the spatial configuration of patches, it is essential to recognize that different organisms may have a different perception of the same landscape. Accordingly, distance-decay relationships should be different depending on the dispersal mode of the organisms because of their different landscape perception (Nekola & White, 1999; Soininen et al., 2007c; Borthagaray et al., 2014). Thus, when considering spatial distance, more actively dispersing taxa should exhibit a smoother decrease in similarities because the organisms would be less affected by barriers and would look for adequate habitats over larger distances (Bush & Whittaker, 1991; Nekola & White, 1999), whereas more passive dispersers would exhibit a greater decrease (Nekola & White, 1999; Steinitz et al., 2006; Astorga et al., 2012). In addition, when considering environmental distances, active dispersers should be more related with an environmental distance than passive dispersers since the former present less dispersal limitations and can better track environmental changes (De Bie et al., 2012). However, dispersal ability not only determines organism landscape perceptions (i.e., connectivity thresholds) but also acts as a key trait when establishing possible environmental controls on organisms (e.g., Beisner et al., 2006; De Bie et al., 2012; Heino, 2013a). All these findings confirm the great importance of organism dispersal ability in determining metacommunity functioning (Damschen et al., 2008), since environmental control, which is a central issue in some of the existing metacommunity paradigms (i.e., species sorting; Leibold et al., 2004), could be minimized due to, for example, high dispersal rates. In turn, these high dispersal rates could be the cause of mass effects, another well-known metacommunity dynamic (Shmida & Wilson, 1985). Therefore, the development of simultaneous studies on several biotic groups (differentiated according to their dispersal abilities) and in the same localities might be a good mechanism for gauging the importance of dispersal against the strength of local conditions (Soininen et al., 2007c) and thus would help to better understand metacommunity functioning. Moreover, dispersal ability also likely determines the degree at which the spatial extent becomes too large to encompass a metacommunity (Heino, 2013a), and this degree is essential to establishing the spatial extent of the study. For many organisms (with the possible exception of unicellular organisms (Finlay, 2002; O'Malley, 2007) but see (Verleyen et al., 2009; Heino, 2010)), the spatial scale that encompasses the metacommunity is likely to be relatively small, usually delimited by physical boundaries (e.g., a drainage basin for stream organisms) (Heino, 2013a). Surprisingly, many distance-decay studies have been conducted at a continental or inter-continental scale (Qian, 2009), with studies rarely considering smaller extents (i.e., under 10 km) (Steinbauer et al., 2012).

Considering all the existing information, we propose a new framework that links distance-decay patterns to different metacommunity dynamics taking into account not only the dispersal ability of organisms but also the spatial extent of the study site (Figure 4.2.1). The aim of the present study is to test the validity of this framework with empirical data obtained from two sites with different spatial extent and to consider several biotic groups that differ in their dispersal abilities. To achieve this aim, we studied the community similarity of three biotic groups with contrasting dispersal strategies (macrofaunal active and passive dispersers and plants) in two pond networks with similar environmental variability (i.e., similar environmental gradients) but different spatial extent (one pond network was 4 times larger than the other pond network). The two pond networks have a relatively small spatial extent (below 10 km), and they are consistent with the natural size of the pond cluster, so the spatial extent of each pond network studied was delimited by an identified physical boundary. We tried to maximize the environmental variability (i.e., environmental gradient) within each study area, considering the broadest available range of pond sizes. Thus, following our framework, we first expected that, in the smaller extent pond network, the decay in community similarity would be steeper than that in the larger pond network (Steinitz et al., 2006; Soininen et al., 2007c; Steinbauer et al., 2012) (see Figure 4.2.1). Second, we also expected that active dispersers would be more related to the environment (i.e., fitting under the expected trend for the species sorting paradigm) (Castillo-Escrivà et al., 2017) than passive dispersers, and thus, we expected a stronger relationship with spatial distance of the latter (Jones et al., 2006; Heino et al., 2014). Consequently, passive dispersers might show a distance-decay pattern closer to the pattern expected under mass effects or neutral model.

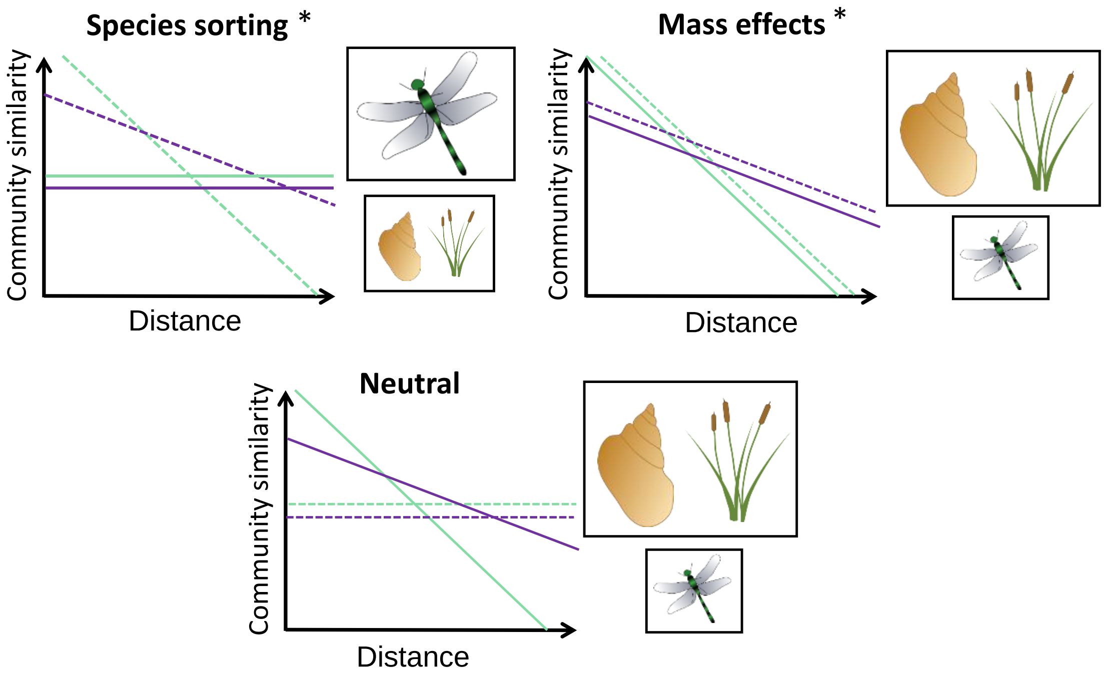

Figure 4.2.1. Conceptual scheme denoting the decrease in community similarity along spatial and environmental gradients taking into account the different types of metacommunity dynamics. The different sizes of organism symbols represent the higher (big symbol) or lower (small symbol) importance of the type of metacommunity dynamics for each biotic group. Purple lines indicate the response in the LEPN (large extent pond network) and green lines in the SEPN (small extent pond network). Solid lines indicate spatial distance, and dashed lines indicate environmental distance. The asterisk denotes the types of metacommunity dynamics that increase in importance at the surveyed small spatial extents (Heino et al., 2014, 2015b). The figure is modified from Heino (2013b). Credits: Symbols courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

METHODOLOGICAL APPROACH

Study sites

For this study we selected two pond networks with similar environmental variability (Ballón et al., 2016) but with different spatial extents: one pond network with a smaller extent (i.e., NW 3) (where the maximum distance between two ponds is 1.4 km) and the other pond network with a larger extent (i.e., NW 4) (where the maximum distance between two ponds is 5.3 km). Thus, the large extent pond network (hereafter LEPN) is almost 4 times larger than the small extent pond network (hereafter SEPN). For the present study, 11 temporary ponds, encompassing a wide range of sizes (from 245 to 78 652 m^2 in SEPN and from 565 to 58 720 m^2 in LEPN), were sampled in each pond network. More information on the study sites is provided in the 'General methodology' section.

Data collection and processing

Environmental and spatial parameters

In this study, the environmental parameters correspond to the water parameters described in the 'General methodology' section and the procedure to obtain them is also described in the mentioned section. We considered as spatial parameters both the distance to the nearest pond (DNP), which was obtained as the straight-line distance between the central point of the studied pond and the central point of the closest pond using Google Maps, and the maximum surface of the different ponds.

Macrofauna and plant sampling

To sample the macrofauna from each pond we followed the procedures described in the 'General methodology' section. The samples were identified to species level whenever possible, except in the case of chironomids, which were identified to subfamily level.

Plant species presence was recorded three times during the season (March, April and May) to identify the highest number of species considering their different growing periods. The surveys were carried out by walking throughout each pond from the outer edge to the centre according to the hydrological gradient surveying all different plant communities. Taxa were identified at species and intraspecific levels.

Data analyses

Community similarity and data matrices

We established three biotic groups with different dispersal strategies: macrofaunal active dispersers (AD), macrofaunal passive dispersers (PD) and plants (PL). All analyses were performed separately for each biotic group within each pond network.

We calculated the similarity in community composition between all pond pairs from each network using the Jaccard dissimilarity with presence-absence data (see Appendix 2 SI Table 2.1-2.6), and then, we removed similarities equal to zero to increase the power of the distance-decay approach (Steinbauer et al., 2012). We had very few similarities equal to zero, which implies an increase in the explanatory power of the regressions and no problem with dissimilarity saturation (Tuomisto et al., 2012). Spatial distances were calculated from the UTM coordinates as Euclidean distances. We used BIO-ENV (Clarke & Ainsworth, 1993) to identify the subset of environmental variables (previously standardized) among all the variables that we had measured (see subsection 'Environmental and spatial parameters') from each site that produced the highest correlation with community similarities. Then, we considered the selected variables in each of the networks. This best subset of variables was then used to calculate the environmental distance matrix based on the Euclidean distances between ponds for each pond network. The R package 'vegan' was used for the BIO-ENV analyses.

Environmental variability assessment

The similarities among the environmental matrices between both pond networks were tested with an analysis of multivariate homogeneity of group dispersions (PERMDISP; Anderson et al., 2006). We conducted the analysis based on Euclidean distances of log-transformed environmental variables except for pH and % of fulvic acids. This test was run to guarantee that only the spatial extent and not environmental variation differed among the pond networks. In addition, a general NMDS considering all the environmental variables from the two pond networks and three specific NMDS considering the subset of environmental variables (selected with the BIO-ENV) for each biotic group were carried out to visually assess variations in the distribution of the ponds from each network. The PERMDISP and NMDS analyses were run using PRIMER v.6.

Environmental versus spatial distance

First, to determine if there was covariation between the environmental and the spatial distances, we performed Mantel tests (with 1000 permutations) between both distance matrices. The Euclidean distances among the samples (environmental, previously standardized, and spatial coordinates) were calculated, separately for each pond network, to obtain the distance matrices that were then correlated with the Mantel test. Our results indicated that there was no significant correlation in any case (see Appendix 2 SI Table 4.2.8). Second, to assess the influence of environmental distance on community similarity given the spatial distance, and *vice versa*, we performed partial Mantel tests (with 1000 permutations), both ranked and non-ranked (i.e., using Spearman and Pearson correlations, respectively). Since we did not find substantial differences in the results between the ranked and non-ranked tests, and all the significant relations that appeared for the ranked version also appeared for the non-ranked, we only show the results from the non-ranked Mantel tests. These analyses were conducted with the R package ‘ecodist’ (Goslee & Urban, 2007).

Environmental and spatial distance-decay

To analyse the distance-decay patterns (both due to spatial distances and to environmental distances), we used regression models. The similarity between the pairs of samples (response variable) was expressed as (1- Δy) with Δy $[0 \leq \Delta y \leq 1]$ being the change in community structure from one pond (*i*=1, … , *N*) to another (*j*= 1, … , *N*), as measured by the Jaccard pairwise dissimilarity measure (Anderson et al., 2011). The Euclidean distance among the samples (environmental, previously standardized, and spatial coordinates) was used as the explanatory variable. We performed three types of regression models according to Soininen et al. (2007c): linear, exponential, and power-law to know which was the best fit in each case. Since we had better fits with the exponential regression model, we only show the results for this model (the remaining models are included in Appendix 2 SI Table 4.2.9). We tested the significance of the regression models using a randomization procedure with 5000 iterations (Logan, 2010). Finally, when the regressions for one biotic group were significant in both networks, we tested the difference in slopes using a permutation procedure with 1000 iterations with the ‘diffslope’ function from the ‘simba’ R package (Jurasinski & Retzer, 2015). Similarly, we also tested the difference in the slopes among the biotic groups of the same pond network.

OUTCOMES

In both pond networks, the pattern of taxa richness among the biotic groups was the same. In the SEPN, the richest group was the PL (74 taxa), followed by the AD (66 taxa) and finally the PD (13 taxa). In the case of the LEPN, the total richness of each group was 102 (PL), 55 (AD) and 27 (PD). With respect to community similarity (Jaccard index), within the SEPN the highest value was 0.833 for the PD, and the lowest value was 0.073 for the PL. Within the LEPN, the AD had the highest value (0.667), and the PD had the lowest value (0.118).

In relation to the measured environmental and spatial parameters, the pond networks have contrasting values of TIC, DIN and % of fulvic acids (Appendix 2 SI Table 4.2.7). However, with the PERMDISP results, we validated that the environmental variability observed in both pond networks is not significantly different (see Figure 4.2.2), although the variables that explain the higher proportion of variability are different at each site (Table 4.2.1). BIO-ENV analyses identified different sets of environmental variables for each biotic group and pond network, although TOC was selected for almost all the cases (Table 4.2.1). The overall correlation with environmental factors was stronger for AD than for PD and PL, independent from the pond network.

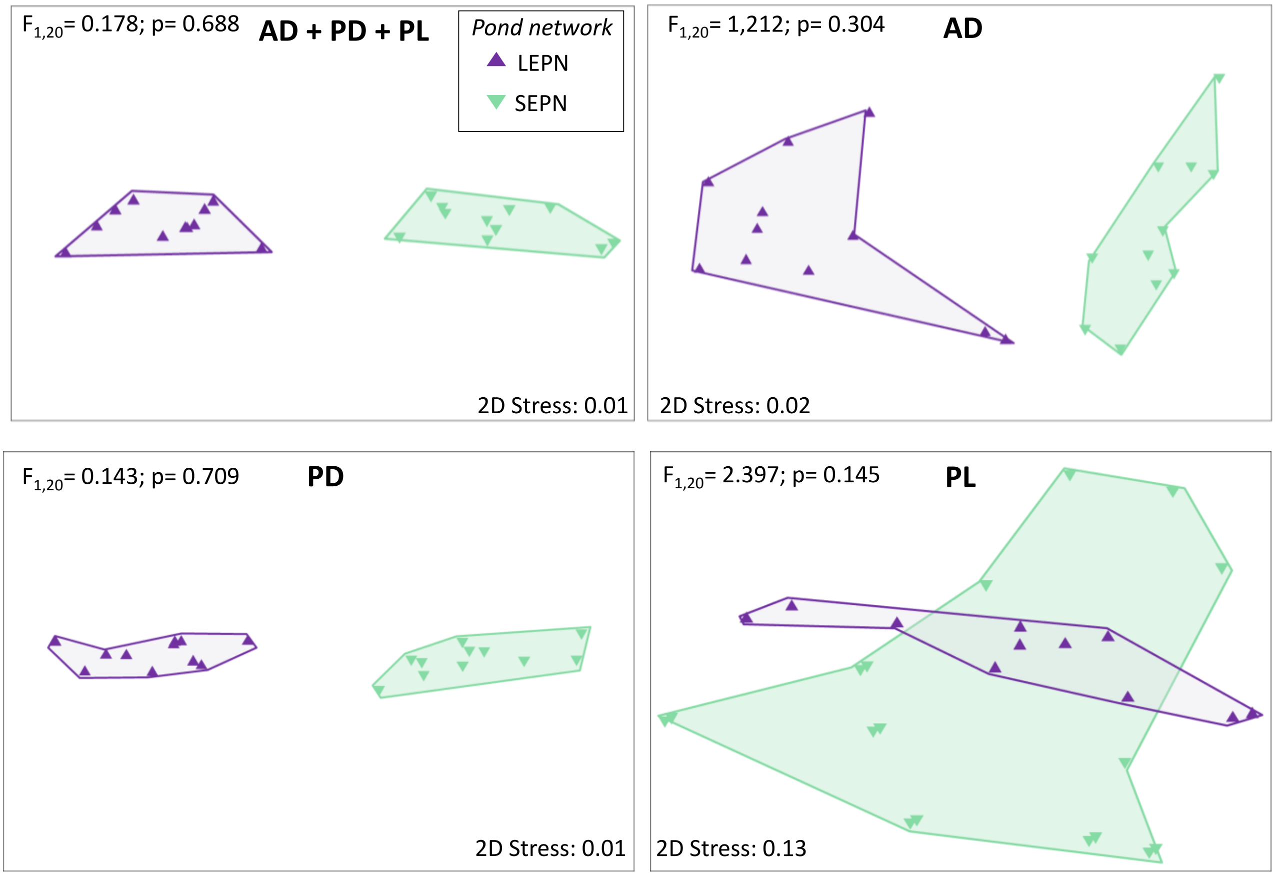

Figure 4.2.2. Non-metric multidimensional scaling (NMDS) ordinations of the ponds considering the whole environmental matrix (Euclidean distances) and the three biotic groups (top, left); considering only the environmental variables with the highest correlation for AD (top, right), PD (bottom, left) and PL (bottom, right) (see Table 4.2.2). PERMDISP results are shown.

Table 4.2.1. Set of environmental variables identified by the BIO-ENV analysis and the overall correlations (Pearson) for each biotic group and pond network. Abbreviations are AD (macrofaunal active dispersers), PD (macrofaunal passive dispersers), PL (plants), SEPN (small extent pond network), LEPN (large extent pond network), TIC (total inorganic carbon), T (temperature), TOC (total organic carbon), TP (total phosphorus), DW (dry weight), and DNP (distance to the nearest pond).

Biotic group	Environmental variables for SEPN	Pearson's R SEPN	Environmental variables for LEPN	Pearson's R LEPN
AD	TIC, pond size	0.627	max. depth, pH, T, TOC	0.722
PD	Conductivity, pH, T, phosphate, TOC, TP	0.455	TOC, fulvic acids, macrophyte DW, pond size	0.701
PL	oxygen, chorophyll-*a*	0.304	Conductivity, TIC, TOC, pond size, DNP	0.477

According to the partial Mantel tests that we performed to evaluate the relative influence of the environmental and spatial distances on community similarity, we observed that community similarity was generally more strongly related to environmental distance when spatial distance was controlled for, than *vice versa* (Table 4.2.2). In fact, spatial distance was not significant in any of the cases. Environmental distance, in contrast, was significant for the AD in both pond networks and for the PD only in the LEPN (Table 4.2.3). Moreover, in the LEPN, the correlation between environmental distance and community similarity was higher for the AD (0.681) than for the PD (0.586) (Table 4.2.2).

Table 4.2.2. Partial non-ranked Mantel correlations between community similarity and environmental distance controlling for spatial distance, and *vice versa*, for each biotic group and pond network. Statistical significance for each partial Mantel correlation value is given in parentheses. Abbreviations are SEPN (small extent pond network), LEPN (large extent pond network), AD (macrofaunal active dispersers), PD (macrofaunal passive dispersers) and PL (plants). Significant differences ($p < 0.05$) are indicated by two asterisks**, and marginally significant differences ($0.1 > p < 0.05$) by one asterisk*.

Distance	Pond network	Biotic group	Mantel r
Spatial (controlling for environmental)	SEPN	AD	0.234 (*p*= 0.064*)
		PD	0.172 (*p*= 0.127)
		PL	0.191 (*p*= 0.088*)
	LEPN	AD	-0.063 (*p*= 0.699)
		PD	-0.030 (*p*= 0.610)
		PL	0.140 (*p*= 0.102)
Environmental (controlling for spatial)	SEPN	AD	0.480 (*p*= 0.024**)
		PD	0.304 (*p*= 0.102)
		PL	-0.116 (*p*= 0.657)
	LEPN	AD	0.681 (*p*= 0.001**)
		PD	0.586 (*p*= 0.005**)
		PL	0.340 (*p*= 0.064*)

Table 4.2.3. Regression parameters for the relationship between community similarity and distance (spatial and environmental) for each biotic group in the SEPN and LEPN. Abbreviations are SEPN (small extent pond network), LEPN (large extent pond network), AD (macrofaunal active dispersers), PD (macrofaunal passive dispersers), and PL (plants). Significant differences ($p < 0.05$) are indicated by two asterisks**.

Distance	Pond network	Biotic group	R^2	p-value	Slope
Spatial	SEPN	AD	0.091	0.014**	$-1.989*10^{-4}$
		PD	0.027	0.119	-
		PL	-0.006	0.420	-
	LEPN	AD	-0.017	0.749	-
		PD	-0.019	0.973	-
		PL	-0.010	0.501	-
Environmental	SEPN	AD	0.257	<0.001**	-0.087
		PD	0.089	0.015**	-0.041
		PL	-0.017	0.783	-
	LEPN	AD	0.457	<0.001**	-0.101
		PD	0.320	<0.001**	-0.159
		PL	0.110	0.008**	-0.081

Similar results were obtained when analysing distance-decay patterns. Thus, spatial distance-decay patterns were significant only for the AD in the SEPN (Figure 4.2.3). In contrast, environmental distance-decay patterns were detected in all cases, except for the PL in the SEPN. No significant differences were found when comparing the environmental distance-decay slopes of the AD and PD (i.e., comparison between groups) and within pond networks (i.e., comparing the same group between the pond networks), which suggests a general environmental distance-decay response of these two biotic groups regardless the spatial extent of the pond network. Within the LEPN, a significant difference in the slopes of the environmental distance-decay for the PD and PL was the only difference detected (*p*=0.041) as it was lower than the slope of the PL (difference in slope PD-PL=-0.0781).

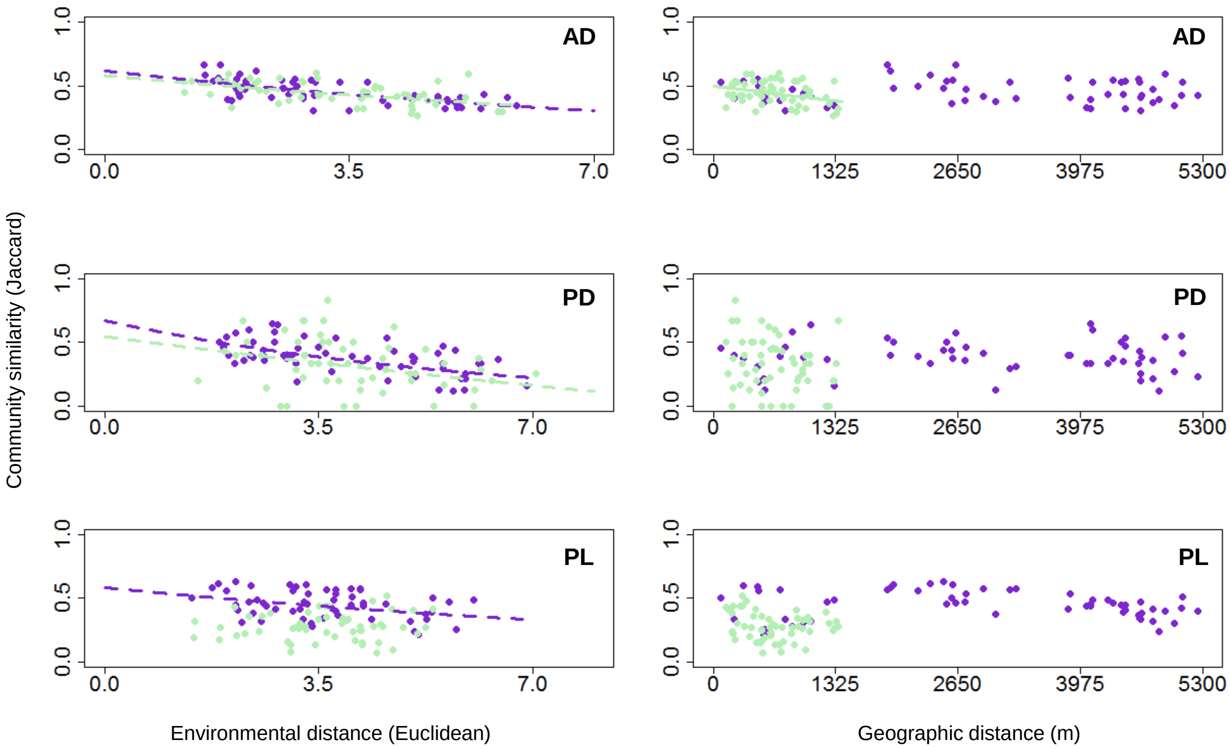

Figure 4.2.3. Relationship between community similarity and environmental and spatial distances for AD, PD and PL. Abbreviations are AD (macrofaunal active dispersers), PD (macrofaunal passive dispersers) and PL (plants). The relationship was best approximated by an exponential model in each case (for regression equations see Table 4.2.3). Only significant relationships are shown. Green dots and lines are the data from the SEPN (small extent pond network) and purple dots and lines from the LEPN (large extent pond network).

DISCUSSION

In accordance with the expected prevalence of species sorting in aquatic systems (Heino et al., 2015a), we found that all biotic groups showed significant distance-decay relationships with environmental distances suggesting that the metacommunities studied had some environmental control. The slopes were consistent for the faunal active and passive dispersers, regardless of the spatial extent of the site, suggesting that the turnover in species composition due to environmental changes is characteristic of each biotic group (at least for the two faunal groups included in the study). In addition to this environmental control of faunal groups, our findings also confirmed the existence of differences in distance-decay patterns among taxa groups depending on their dispersal strategies and the spatial extent of the pond network (Tuomisto et al., 2003; Thompson & Townsend, 2006; Soininen et al., 2007c; Grönroos et al., 2013). In this sense, we expected that spatial distance would be more important than environmental distance for the two passively dispersing groups (i.e., PD and PL) due to their likely dispersal limitations (Nekola & White, 1999), but we did not find any significant relationship. The partial Mantel tests indicated that an effect of spatial distance on community similarity was absent. Moreover, the distance-decay relationships were also consistent with this finding, reinforcing the concept that spatial distance plays a very weak role in comparison to environmental distance in these metacommunities. This result could be attributed to an absence of dispersal limitations in the vectors implied in passive dispersal since we observed an absence of dispersal barriers in the two pond networks studied. The lack of a significant relationship with spatial distance for the passive dispersers (PD and PL) reinforces the concept of a strong species sorting effect ruling their metacommunities. In contrast, the AD showed spatial distance-decay but only in the pond network with the smaller spatial extent. This result is consistent with the higher importance of mass effects over other metacommunity dynamics at smaller spatial extents (Heino et al., 2014), and our results indicate that this mass effects is more evident for the active dispersers than for the passives. When comparing among the groups within the same pond network, there were no significant differences in their slopes except between the PD and PL in the LEPN. Moreover, it is important to note that the existence of different patterns in the same group between pond networks could be related to a difference in the strength of the environmental gradient between the sites (Nekola & White, 1999; Heino et al., 2015a). However, in our study, we might reject this possibility as both pond networks had similar environmental distances and environmental variability. Therefore, although the length of environmental gradients usually increases with an increasing spatial extent (Heino et al., 2015a), we did not find this effect. Thus, our results are consistent with the concept that different biotic groups with differential dispersal abilities can respond similarly to the same environmental gradients (Heino, 2010).

We confirmed that the organisms with different dispersal modes have different responses to the spatial extent of the pond network (Heino et al., 2014). According to previous studies (Jones et al., 2006; Steinitz et al., 2006; Soininen et al., 2007c; Steinbauer et al., 2012), we expected steeper slopes in the SEPN than in the LEPN. However, we were not able to accept or reject this hypothesis since we only found one spatial distance-decay relationship. Our results indicated some role of spatial distance on the active dispersers, and not on the passive taxa (either PD or PL), that was only detected at the smaller spatial extent since the group presented a significant decline in similarity with an increasing spatial distance. It is possible that we did not detect the effect of spatial distance in the LEPN due to the dominance of species sorting (i.e., environmental control) since species sorting appears to be the most important mechanism structuring communities at various spatial extents (Heino et al., 2015a).

However, we are aware that despite the different spatial extents of the studied networks (the LEPN is almost 4 times larger than the SEPN), the maximum extent of the larger pond network is quite small (< 6 km) compared with the distances in other studies (Briers & Biggs, 2005; Thompson & Townsend, 2006; Astorga et al., 2012). In fact, most of the studies on the spatial decay of community similarity have considered very large extents (e.g., Van De Meutter et al., 2007; Morlon et al., 2008; Heino, 2010) only a few of the studies focused on areas with small spatial extents (e.g., Jones et al., 2006; Soininen et al., 2007a; Girdler & Barrie, 2008). We agree, then, with Steinbauer et al. (2012) on the concept that more studies at ecosystems with smaller spatial scales are needed to find a more general conclusion. Furthermore, Heino (2013b) highlighted the more realistic approach of studying metacommunities within small spatial extents than across larger extents since metacommunity dynamics are more likely to act within ecologically defined regions.

Since both pond networks are at similar latitudes and their environmental variability is not significantly different, we might discard these factors as possible explanations for the differences found (Soininen et al., 2007c; Qian & Ricklefs, 2012; Kneitel, 2016). In addition, the fact that environmental and spatial distances were independent makes the results truly reliable when detecting a distance-decay relationship (Nekola & White, 1999; Thompson & Townsend, 2006). In addition, we have also avoided problems related to comparisons between studies that were differently designed because we followed the same sampling design for the biotic groups in both networks, and we also resolved the ‘sampling effort’ effect despite the pond size gradient (Steinbauer et al., 2012). As we already noted, there are few studies that have analysed the distance-decay relationship at small spatial extents. Thus, our study is important since it tries to increase the knowledge on this issue at small spatial extents, and this study highlights the concept that the type of metacommunity dynamics ruling community similarities is strongly influenced by spatial extent (Heino et al., 2014). It is highly likely that our results differ from those in studies that performed similar analyses because they were conducted at larger spatial extents. Furthermore, it is important to note that studying two small pond networks ensured that we studied one single metacommunity in contrast with the studies that encompass very large spatial extents (Heino, 2013a). Although similar studies from ponds also consider the species sorting as the main dynamic structuring the communities (e.g., Hill et al., 2017), we can not discard the possibility that the patterns observed and the underlying mechanisms are context dependent (e.g., Tonkin et al., 2016) since we only studied two pond networks. In line with this, there are regional factors that may differ among sites that we did not consider in our study. These factors are, for instance, the effect of a prevailing wind direction (Horváth et al., 2016) that may, in turn, have a different effect on active and passive dispersers, or the differences among sites regarding the species that may act as vectors of dispersion such as mammals, waterbirds or amphibians (e.g., Bohonak & Whiteman, 1999; Figuerola & Green, 2002; Vanschoenwinkel et al., 2008).

Finally, the group of PL from the SEPN was the only group that did not show a significant decay pattern. This result could be explained by the fact that plant communities likely disperse over scales that exceed those in this pond network, and hence, despite the existence of an environmental gradient, the effect of this gradient remains masked by the high exchange between the ponds. In contrast, previous studies did find a negative relationship between similarity and distance for different types of plant communities. However, most of the studies were conducted at very large spatial extents (e.g., Nekola & White, 1999; Warfe et al., 2013; Nekola & McGill, 2014; König et al., 2016).

In conclusion, our results show that the metacommunity dynamics occurring in each pond network were different and that although mass effects are usually the prevailing mechanism at small spatial extents (Heino et al., 2014), we found evidences of greater importance from other metacommunity dynamics. Nevertheless, we tried to link one metacommunity dynamics with the patterns found for each biotic group studied, and we were aware that some authors considered this a misconstruction since they encourage future investigations to cover the full spectrum of metacommunity theory (Qian & Ricklefs, 2012; Heino et al., 2014). Thus, we wanted to highlight the prevalence of one type of metacommunity dynamics over each biotic group but we did not overlook the remaining metacommunity dynamics since it is likely that they are all playing interactive roles (Leibold et al., 2004). In the case of the LEPN, the three groups studied seem to follow the idea of species sorting perspective indicating that environmental conditions are mainly responsible for structuring these metacommunities independently of organism dispersal modes. This result is consistent with other studies indicating that species sorting prevails in metacommunities over other mechanisms (Poulin, 2003; Briers & Biggs, 2005; König et al., 2016). Hence, in the LEPN, communities appeared to be homogenized by dispersal to a degree (Van De Meutter et al., 2007), while in the SEPN, each group was likely to be mainly driven by a different mechanism: mass effects for the AD, species sorting for the PD and a pattern that was difficult to associate with any metacommunity mechanism for the PL. Therefore, our results demonstrate the importance of studying metacommunity dynamics and distance-decay at smaller spatial extents since we found differences with respect to studies performed at larger spatial extents and between pond networks that cover a small spatial extent.

CHAPTER 3

Disentangling the effects of network connectivity on biodiversity patterns in macroinvertebrate metacommunities

OVERVIEW

One of the major goals of community ecology is to understand the mechanisms underlying biodiversity patterns at local and regional scale. The application of graph theory to metacommunity ecology allows a deeper analysis of the network structure effect on biodiversity patterns. In this study, the structure of the pond network was analyzed by means of individual metrics for each patch (three centrality metrics: ‘degree’, ‘closeness’ and ‘betweenness’), and descriptors of the whole network structure (three network properties: ‘linkage density’, ‘connectance’ and ‘diameter’), having one value per network. Then, we tried to link the macroinvertebrate biodiversity with the network metrics of four networks of Mediterranean temporary ponds. Biodiversity was described by calculating 1) regional biodiversity metrics, which take into account the rest of the ponds of the network, and 2) local biodiversity metrics, which characterize the faunal composition of each pond. The environmental characteristics of the ponds, which included habitat condition, water characteristics and pond size were also included in the analysis, since they have been reported in previous studies as main drivers in community assembly. We analyzed the relationships between biodiversity metrics as the response variables, and pond characteristics and network metrics as the explanatory ones. Most of the biodiversity metrics were not explained by environmental characteristics or network metrics alone, but through a combination of them both. Our results showed that different network metrics which measure connectivity at different spatial scales within the network (from a neighboring scale -degree- to a broader scale -closeness-), affect the biodiversity value of the ponds at both regional and local level. The environmental characteristics of the ponds played a significant role, especially highlighting the role of pond size over the other environmental variables considered. Overall, regional biodiversity metrics showed similar patterns in all the networks studied mainly in response to connectivity, whereas local biodiversity metrics showed greater network-dependent patterns, mostly in response to environmental characteristics.

BACKGROUND

Metacommunity theory relates local scale patterns of species diversity with regional scale processes (O'Dwyer & Green, 2010). Within this framework, dispersal has been pointed as the responsible of transferring the effects of regional heterogeneity to the local scale and can lead to a progressive homogenization of the metacommunity (Chase, 2003). However, the proximity among localities within a region, as well as other landscape attributes (for instance, barriers), can influence dispersal rates (e.g., Germain et al., 2019). Hence, the potential connections at network level among the communities can shape the metacommunity structure, and so within spatial biological theory, isolation of a patch or community has been considered an essential issue (Economo & Keitt, 2010). Therefore, the exchange of individuals by dispersal between communities inhabiting distinct patches of a network is highly determined by its connectivity and is what determines the metacommunity functioning (Hubbell, 2001; Leibold et al., 2004). Indeed, connectivity is especially important since it can limit the movement of genes, individuals or populations among patches (Erős et al., 2012; Gonzalez et al., 2017), affecting at different scales and presenting different functions (Noss, 1991; Tischendorf & Fahrig, 2000).

The application of graph theory to landscapes, and by extension to metacommunity ecology, represents an important advance in the area of spatial modeling and permits the study of networks from a purely spatial view (Bunn et al., 2000; Urban & Keitt, 2001; Estrada & Bodin, 2008; Urban et al., 2009). Thus, graph theory provides a robust procedure for the quantification of metacommunity network structure and local community isolation (Borthagaray et al., 2015b). The number of empirical studies dealing with the role of habitat connectivity in metacommunities is quite low in comparison with the number of theoretical studies (but see Staddon et al., 2010; Carrara et al., 2014; Medina Torres & Higgins, 2016). Within theoretical studies, it is very frequent to apply a neutral model to evaluate the change in metacommunity diversity with changes in connectivity. These studies have shown that the spatial arrangement of patches has considerable impacts on the structure at both local and regional scale (Economo & Keitt, 2008, 2010; Desjardins-Proulx & Gravel, 2012), and consider some concepts of the classical island biogeography theory, which emphasizes the importance of island isolation on local diversity (MacArthur & Wilson, 1967). Considering, then, island, or patch isolation, as a measurable concept and the inverse of centrality, the most common network centrality metrics extracted from a graph analysis provide us with a scale of patch isolation within the landscape network from local neighborhood to global (Estrada & Bodin, 2008; Economo & Keitt, 2010). For instance, 'degree centrality' corresponds to the flux of dispersal directly entering a node from other nodes and is dependent, then, on the local neighborhood of a node, whereas 'closeness centrality' measures a global position of a node in the network since it represents how close a node is to all other nodes (Borthagaray et al., 2015b). These metrics of network centrality are a good tool for measuring landscape connectivity.

Theoretical studies, mainly using dendritic networks, that analyze the relationship between connectivity and biodiversity at local scale (i.e. species richness), observed higher species richness in the more connected sites within the network rather than in the less connected ones (e.g., Muneepeerakul et al., 2008; Economo & Keitt, 2010; Seymour et al., 2015). A priori, this observation could be also expectable in systems with no directional connectivity (e.g. urban ponds; Gledhill et al., 2008; Hill et al., 2017). In fact, Chase (2003) observed that more connected pond communities had higher species richness than less connected communities, indicating thus a similar pattern in pond networks to river networks. However, species richness only represents a facet of local biodiversity, and a more complete view could be obtained using also other complementary biodiversity metrics (Heino et al., 2005; Gascón et al., 2009a; Hassler et al., 2010; Gallardo et al., 2011; Jarzyna & Jetz, 2016). For instance, when analyzing the effect of isolation on biodiversity it is especially interesting to include some kind of phylogenetic relatedness indices since they capture the evolutionary relationships among species coexisting in an assemblage whereas other biodiversity metrics do not (Shimatani, 2001; Heino et al., 2005). Indeed, a higher overdispersion of species per genus is expected in more isolated sites (Simberloff, 1970; Brewin et al., 2009). Therefore, isolation may not only affect biodiversity at "species" level but also at phylogenetic level. For instance, taxonomic distinctness, could be considered as a proxy of community's phylogenetic structure and highlight both the similarities and differences of co-occurring species (Clarke & Warwick, 2001; Webb et al., 2002). Although this relationship has been rarely analyzed, some studies have found an increment of taxonomic distinctness but a decrease of average taxonomic distinctness with isolation (Brewin et al., 2009; Marcantonio et al., 2013; Quiroz-Martínez & Salgado-Maldonado, 2013).

When moving towards a regional effect of connectivity, Chase (2003) observed that more connected pond communities had lower contribution to regional species richness than less connected communities. In the same line, Mouquet & Loreau (2003) also emphasized the role of dispersal to progressively homogenizing communities, implying a reduction of beta diversity. In order to improve the knowledge of the mechanisms that are driving regional biodiversity, a very useful approach to partition the total beta diversity in sites and species contributions was proposed some years ago by Legendre & De Cáceres (2013). The advantage of this approach is that a single value of the contribution of a site to the total beta diversity can be obtained (LCBD, local contribution to beta diversity), i.e., a measure of the uniqueness of a site, as well as the species contribution to beta diversity (SCBD). Some recent studies using the just mentioned metrics observed the influence of not only the spatial predictors, but also of the environmental predictors as drivers of beta diversity (Tonkin et al., 2015; Heino & Grönroos, 2017; Hill et al., 2017; da Silva et al., 2018; Landeiro et al., 2018). However, these studies although took into account spatial variables, did not explicitly evaluate the possible effect of the isolation of the sites within the network on beta diversity and its components despite it has been suggested that higher isolation would be related with greater beta diversity (Borthagaray et al., 2015b; Jamoneau et al., 2018).

Additionally, apart from connectivity there are also some local characteristics that have long been considered key to modulate biodiversity. In this sense, habitat quality could be determinant (Hodgson et al., 2011; Thornton et al., 2011; Altermatt & Holyoak, 2012), as well as the morphology of the basin or the vegetation growing in it (Williams et al., 1999; Olden et al., 2001). According to several studies regarding very different types of habitats, habitat size is positively related to species richness and diversity (King et al., 1996; Spencer et al., 1999; Hillebrand & Blenckner, 2002; Magioli et al., 2015; Mohandass et al., 2017) although other works did not find that relationship (e.g., Snodgrass et al., 2000; Savage et al., 2011). In relation to water characteristics, previous studies reported a negative effect on species richness (Jeffries, 1991; Brodersen et al., 1998) and more recently it has been proven that the components of beta diversity can change with water chemistry (Winegardner et al., 2017; García-Girón et al., 2019). In fact, Hodgson et al. (2011) in an exhaustive review suggested a bigger effect on biodiversity of variations in habitat area and quality rather than of variations in connectivity. Thus, when trying to analyse biodiversity drivers within a metacommunity framework, patch local characteristics must not be disregarded. In line with this, Hill et al. (2017) examined the influence of both spatial (distinguishing local and overall scales) and environmental variables on beta diversity and its components, finding a greater importance of the environmental rather than spatial variables, and within the latest, a greater role of the local rather than overall spatial variables.

The present study examined how the network connectivity determines biodiversity patterns in metacommunities but taking also into account the potential role of patch local characteristics. To do so, we studied 4 different pond networks, taking advantage that networks of lentic waterbodies are excellent systems in where to study the connectivity effect on biodiversity patterns, since: 1) are good examples of patchy habitats being separated by a terrestrial matrix; 2) are abundant and therefore, pond network can include a wide gradient of connectivity, i.e., from isolated to highly connected ponds; 3) each pond encloses a discrete community that could be easily compared to the neighboring pond communities; and 4) local and regional scale could be easily separated, local referring to pond and regional to the pond network (Blaustein & Schwartz, 2001; De Meester et al., 2005; Williams, 2006). Thus, in all pond networks we evaluated the centrality of the patches within the network by calculating different network centrality metrics. Moreover, we considered several biodiversity metrics in order to cover different facets of biodiversity (Heino et al., 2005; Gascón et al., 2009; Gallardo et al., 2011), as well as different spatial scales, from a local to a regional biodiversity spectrum. We then investigated whether the patterns in biodiversity both at local and regional level are driven by patch local characteristics or network centrality metrics (covering the gradient from a neighboring to a global scale within the network) or by a combination of them. Firstly, we would expect a negative relationship between patch centrality and the regional biodiversity metrics (i.e., beta diversity and its components) since increasing connectivity among patches in a network would homogenize species composition among ponds, decreasing, then, community dissimilarity (Chase, 2003; Mouquet & Loreau, 2003; Jamoneau et al., 2018). Secondly, according to Hill et al. (2017), we would expect a greater effect of the environmental characteristics of the ponds rather than network centrality metrics on beta diversity and its components. Thirdly, within the network centrality metrics, degree centrality (which evaluates connectivity at neighboring scale) would largely drive beta diversity and its components rather than closeness centrality (which evaluates connectivity at regional scale). Fourthly, less centrality (i.e., an increase in isolation) would promote the increment of the taxonomic relatedness of species, but a decrease in average taxonomic distinctness in accordance with previous studies (Brewin et al., 2009; Marcantonio et al., 2013; Quiroz-Martínez & Salgado-Maldonado, 2013). Finally, according to theoretical studies (Economo & Keitt, 2010), we would expect a positive relationship between the centrality of the patch in the network and alpha diversity since dispersal would be facilitated in more central patches.

METHODOLOGICAL APPROACH

Study sites

We used the information on the macroinvertebrates assemblages of the four pond networks (NW 1, NW 2, NW 3 and NW 4) described in the 'General methodology' section (see Appendix 3 SI Table 4.3.6).

Sampling

To sample the macroinvertebrates and to obtain the water parameters from each pond we followed the procedures described in the 'General methodology' section. From all the water parameters listed, for this study we selected the following ones: maximum depth, dissolved oxygen, conductivity, pH, temperature, DOC (dissolved organic carbon), TOC (total organic carbon), phosphate, TP (total phosphorus), DIN (dissolved inorganic nitrogen), TN (total nitrogen), fulvic acids, macrophyte biomass, planktonic chlorophyll-*a*, molar DIN / molar TP, and absorbance at 440 nm.

Networks metrics

The whole set of water bodies configuring each network was determined by aerial photographs from Google Earth. To stablish the total area in which we looked for waterbodies, we created a polygon connecting the most external sampled ponds, and then we constructed an outer polygon using the maximum distance between the two more distant sampled ponds as a buffer distance. This outer polygon gives us the total area to be scanned looking for other water bodies that finally conformed the network. From now on, we will refer to the area of that outer polygon as SEA (i.e., network spatial extent measured as an area; Heino et al. 2014), which was extracted from Google Earth Pro. Then, in order to obtain the structure of the network, we used the percolation distance (PDist) which is the minimum distance at which all water bodies of the network are connected (Urban & Keitt, 2001; Rozenfeld et al., 2008). We did this for the four studied networks (Figure 4.3.1).

As descriptors of the network structure we calculated: 'linkage density', 'connectance' and 'diameter'. In our context, linkage density is defined as the average number of realized links per water body (Borthagaray et al., 2015b). Connectance is the number of connections or links realized divided by the maximum possible number of links (Williams et al., 2002; Newman, 2003; Proulx et al., 2005). Finally, the diameter of a network represents the number of steps necessary for movement through the whole network (Urban & Keitt, 2001; Newman, 2003). Besides, we also estimated the spatial extent of the network as the maximum distance between the two furthest water bodies moving through the patches and not as a straight-line (hereafter, SED, i.e., network spatial extent measured as a distance; Heino et al., 2014). These metrics were computed with the 'igraph' package in R (Csárdi & Nepusz, 2006).

Besides the characterization of the network, and once obtained the percolation distance, we calculated for each water body three metrics that focus on different concepts and definitions of centrality: 'degree centrality', 'closeness centrality' and 'betweenness centrality' (Economo & Keitt, 2008; Borthagaray et al., 2015b). Degree centrality is the number of direct links between water bodies; closeness centrality calculates how close a water body is to the rest, and betweenness centrality quantifies how frequently a water body is on the shortest path between every possible pair of the other water bodies. These three metrics were calculated with the R package 'sna' (Butts, 2016).

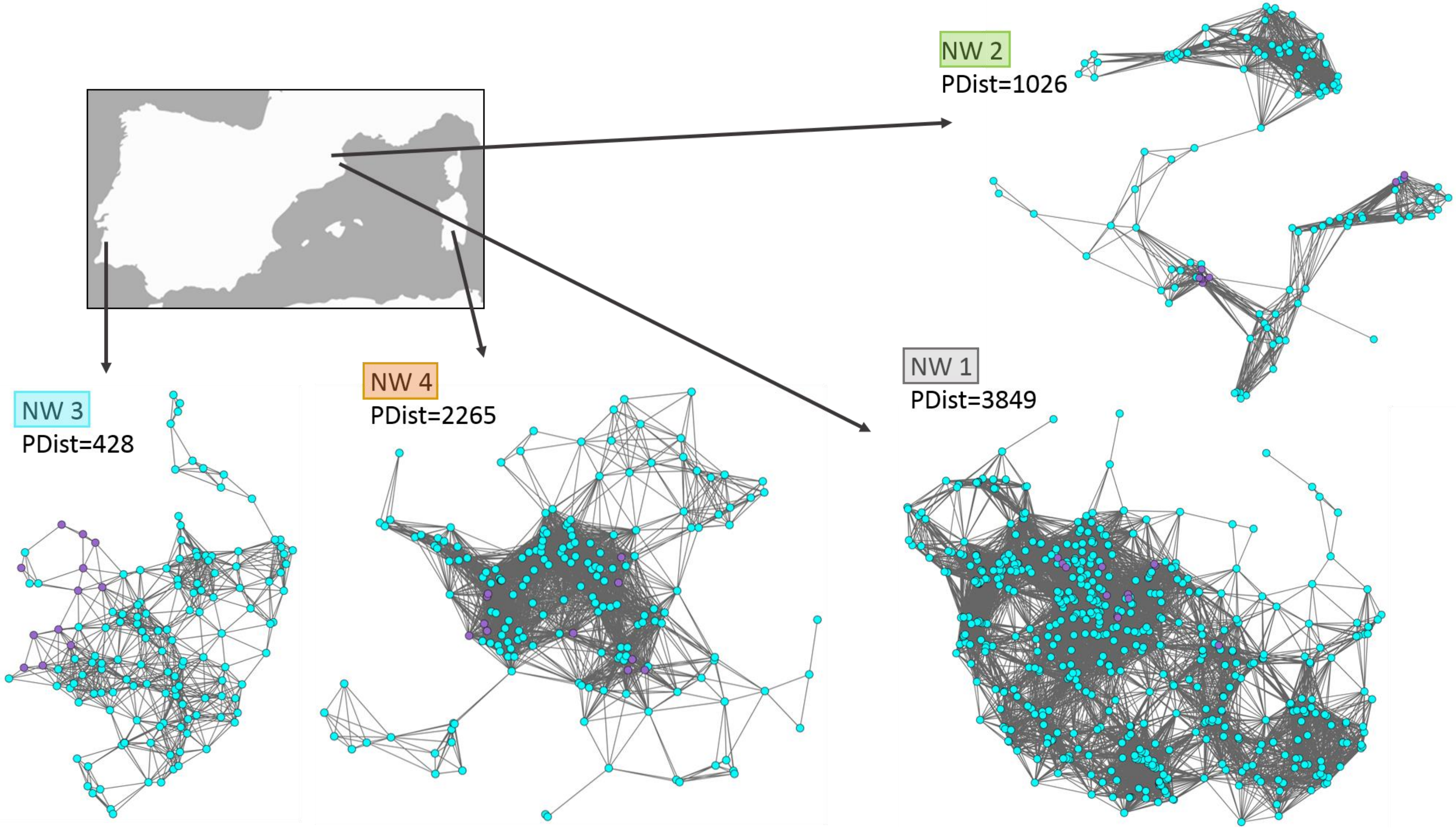

Figure 4.3.1. Location map of the four networks. The circles in purple correspond to the sampled ponds (between 10 and 12 in each network) and the turquoise circles are the rest of the waterbodies that encompass the network. Each network is obtained by linking the pairs of patches that reach the minimum distance linking the whole patch system, i.e., the percolation distance (PDist), which is expressed in meters.

Biodiversity metrics

At network level (meaning one value for each network) and with the information obtained from the sampled ponds, we calculated the value of beta diversity and the partition of the total beta diversity (BD) into replacement (BD-re) and abundance difference components (BD-ad). To do so, we used the Podani-family decomposition of the Jaccard dissimilarity coefficient in the quantitative form (Legendre, 2014). To assess the individual contribution of the species to the beta diversity, we computed SCBD indices (Species Contribution to Beta Diversity) on Hellinger transformed abundance data following Legendre & De Cáceres (2013). Then we used the SCBD values to obtain a proxy of species uniqueness per network (PSSAM), calculated as the percentage of species that presented a value of SCBD above the mean value of the sampled ponds.

At pond level (meaning one value for each pond) we calculated two groups of metrics. The first group of metrics takes into account the rest of the ponds of the network (i.e., beta diversity approach; hereafter we will refer to them as "regional biodiversity metrics") and the other one characterizes the fauna composition of each pond (hereafter we will refer to these metrics as "local biodiversity metrics"). As regional biodiversity metrics we calculated the 'local contribution to beta diversity' (LCBD) and its decomposition into replacement (LCBD-re) and abundance difference (LCBD-ad) components. The LCBD-re and LCBD-ad measure how exceptional each pond is, when compared to other ponds within the same network, in terms of replacement or abundance difference of species. We also calculated SCBD-prop, which is the proportion between the number of species with a value of SCBD above the mean value of the set of sampled ponds in a network to the total species richness of the pond. As local biodiversity metrics we estimated the alpha diversity by means of two approaches: as the rarefied species richness (S-rar), considering the minimum abundance of individuals obtained in a pond within the same network, and as the Shannon-Wiener diversity index (H) per pond. Finally, we calculated two different indices of taxonomic diversity; one that uses abundance data as it is the taxonomic distinctness (TD) and one that uses presence/absence data as it is the average taxonomic distinctness (ATD). We considered branch lengths unweighted and hence, the steps between each taxonomic level in the tree are considered to be equal (Clarke & Warwick, 1998, 2001).

Total beta diversity (BD) and its decomposition, as well as SCBD and LCBD and its decomposition, were obtained using the functions 'beta.div.comp', 'beta.div' and 'LCBD.comp' from package 'adespatial' in R (Dray et al., 2018). Species richness rarefaction was done using the function 'rarefy', and the indices of taxonomic distinctness were calculated using the function 'taxondive', all from package 'vegan' (Oksanen et al., 2018).

Data analyses

To disentangle whether the network descriptors were redundant or not among them, we performed Pearson correlation analyses. In order to validate that all the networks showed similar variability in water characteristics (previously standardized) a PERMDISP analysis was done, using the function 'betadisper' from 'vegan' package (Oksanen et al., 2018). According to this analysis, the variability of water characteristics among the sampled ponds of the different networks was not significantly different ($F_{3,41}$=0.033; *p*=0.990). In order to sum up the variability of water characteristics of the sampled ponds and posteriorly use it as an explanatory variable in regression analyses, a principal component analysis (PCA) was performed with the above mentioned water variables. These variables were previously standardized separately by each network. Considering the scree plot criteria only the first principal component axis (PC1; explaining 25.1 % of the total variability) was relevant, so we kept this component for posterior analyses. The PC1 (SI Figure 4.3.1) was correlated significantly (p<0.05) and positively with dissolved oxygen (r= 0.509), temperature (r= 0.401), pH (r= 0.377), molar DIN / molar TP (r= 0.333), and negatively with absorbance at 440 nm (r= -0.848), TN (r= -0.816), TOC (r= -0.813), DOC (r= -0.752), TP (r= -0.616), planktonic chlorophyll-*a* (r= -0.402) and phosphate (r= -0.311).

To analyse the relative importance of 1) the network centrality metrics, 2) the water characteristics, 3) habitat condition (ECELS index), and 4) the pond size as drivers of biodiversity, we performed multiple regression analyses. We used beta regression models for the regional biodiversity metrics (i.e., LCBD, LCBD-re, LCBD-ad, and SCBD-prop), since these response variables showed values between 0 and 1 (i.e., were beta-distributed; Cribari-Neto & Zeileis, 2009). On the other hand, we used linear models for the local biodiversity metrics (S-rar, H, TD and ATD). The explanatory variables (previously standardized separately for each network except PC1) were PC1, pond size, closeness, degree, betweenness and ECELS. We included the network identity as a factor, and its interaction with the explanatory variables to identify the possible existence of differential responses linked to network intrinsic differences. To identify the relevant explanatory variables, we performed an automated model selection based on Akaike's information criterion for small sample sizes (AICc) (Barton, 2017). Then, we generated a subset of models with $\Delta AICc \leq 2$ and finally, from that subset, we chose as the 'best model' the one with the highest pseudo-R^2 (in the case of the beta regression) or adjusted-R^2 (in the case of the linear models). The model selection procedure was performed with the package 'MuMIn' (Barton, 2017).

OUTCOMES

Pond networks characteristics: connectivity and biodiversity

Our results showed that networks with the smaller percolation distance (Table 4.3.1) were also the ones with lower spatial extent distance (i.e., significantly correlated with SED; Figure 4.3.2). Interestingly, connectance and diameter were not significantly correlated with any other network descriptor indicating, hence, their complementarity. Nevertheless, linkage density was significantly and positively correlated to the percolation distance and the network extent (both SEA and SED) (Figure 4.3.2). Using these complementary network structure metrics, the studied networks can be characterized as follows (Figure 4.3.1 and Table 4.3.1): from a very dense and big network (i.e. with the greatest SEA value and linkage density, NW 1) to a sparser and smaller network (i.e. having the largest diameter but the smallest connectance and linkage density, NW 3), and the others including in-between characteristics (i.e. a small network with medium connectance and linkage density, NW2, but also a larger one, with higher connectance and linkage density, NW4). On the other hand, in all the networks, the values of total beta diversity were not high, ranging from 0.342 to 0.413 (thus always below 0.5; Table 4.3.1). Interestingly, in all the networks the replacement component (BD-re) explained more than 80% of the observed total beta diversity (BD), leading a residual contribution to the abundance difference component (BD-ad) (being always below 20%; Table 4.3.1).

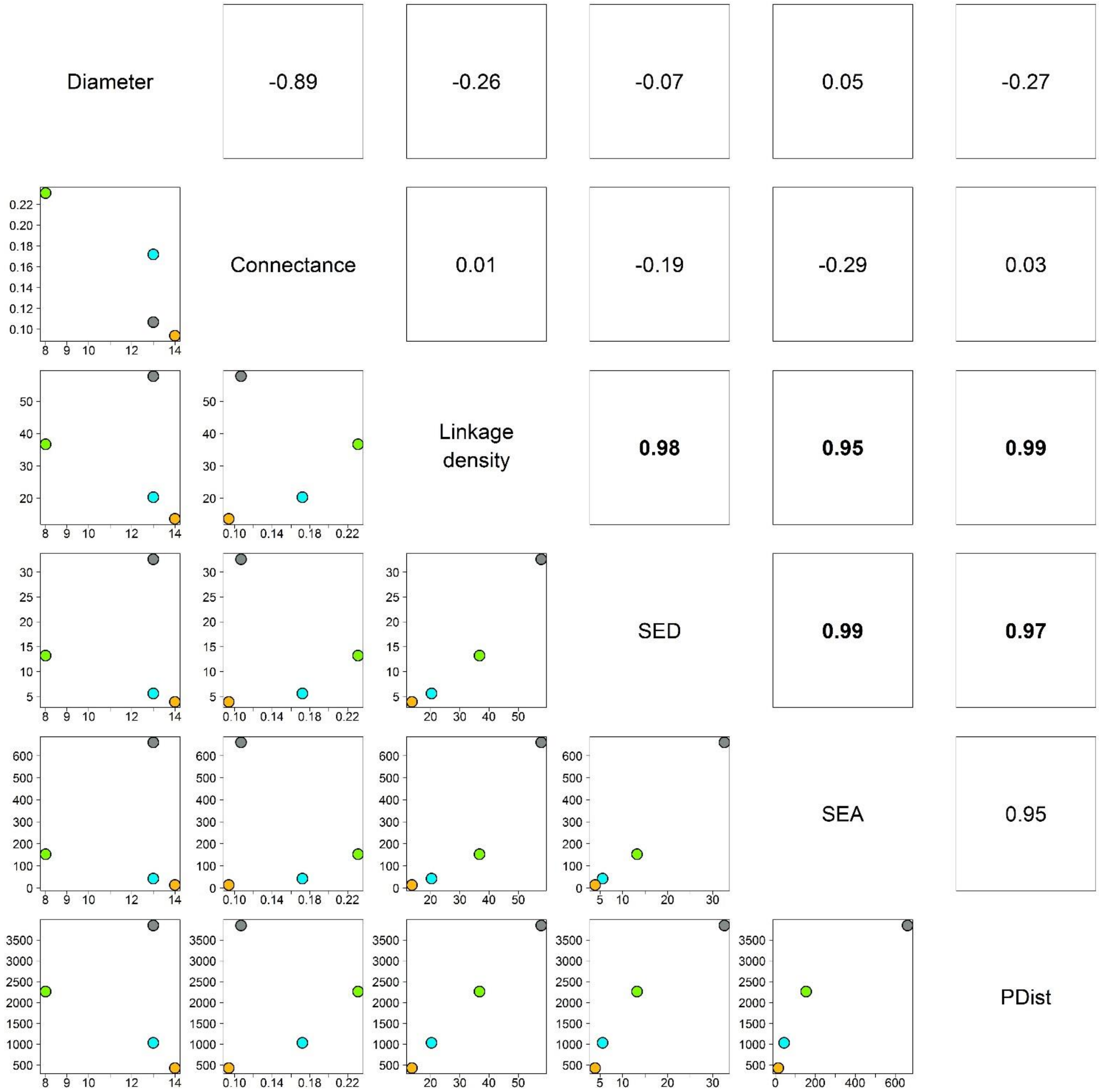

Figure 4.3.2. Resulting plot from the Pearson correlation analyses among the network metrics. Abbreviations are SED (spatial extent distance), SEA, (spatial extent area) and PDist (percolation distance). Significant correlations are indicated in bold. N=4.

Table 4.3.1. Main parameters from networks characterization. Abbreviations are PSSAM (percentage of species that presented a value of SCBD above the mean value of the sampled ponds), BD (total beta diversity), BD-re (replacement component of beta diversity), BD-ad (abundance difference component of beta diversity), BD-re/BD (proportion of beta diversity explained by replacement), BD-ad/BD (proportion of beta diversity explained by abundance difference), SEA (spatial extent area), SED (spatial extent distance) and PDist (percolation distance).

Network	Biodiversity metrics							Network descriptors						Ponds descriptor
	Mean rarefied species richness	PSSAM	BD	BD-re	BD-ad	BD-re/BD	BD-ad/BD	SEA (Km^2)	SED (Km)	PDist (m)	Diameter	Connectance	Linkage density	Pond size range (m^2)
NW 1	26.109	24.031	0.413	0.357	0.057	0.863	0.137	661	32.591	3849	13	0.107	57.816	24-54920
NW 2	19.871	25.758	0.399	0.349	0.050	0.874	0.126	42.600	5.578	1026	13	0.172	20.303	29-2015
NW 3	21.676	16.484	0.346	0.300	0.046	0.866	0.134	13.200	3.892	428	14	0.094	13.603	245-78652
NW 4	30.500	21.591	0.342	0.286	0.056	0.837	0.163	153	13.226	2265	8	0.231	36.725	565-79990

Biodiversity drivers

The subset of models with ΔAICc$\leq$2 showed that regional biodiversity values were intrinsically distinct among networks (see the network identity's importance alone and also in the interaction terms; Figure 4.3.3A and SI Table 4.3.1). Besides these intrinsic differences, our models also indicate a high importance of two centrality metrics (degree and closeness), the habitat condition (ECELS), and pond size for the regional biodiversity. Similarly, the intrinsic differences among networks were also evident for the local biodiversity metrics (Figure 4.3.3B), and again, some centrality metrics (mainly closeness and in a minor way, degree) were important for local biodiversity values, while the importance of habitat condition decreased.

When looking at the 'best models' (i.e., the ones with highest pseudo-R^2 or adjusted-R^2) for each of the regional biodiversity metrics, intrinsic different values for each network were detected for LCBD, LCBD-re and SCBD-prop. Thus, the smallest network with least connectance and linkage density (i.e., NW 3) showed the lowest values of LCBD, LCBD-re and SCBD-prop. The biggest and most dense (highest linkage density) network (i.e., NW 1), only showed the highest values for SCBD-prop, whereas the highest values of LCBD and LCBD-re were found in a smaller network but with medium connectance and linkage density (i.e., NW 2) (Figure 4.3.4; SI Table 4.3.2). Besides these intrinsic differences among networks, we also found general relationships. For instance, both LCBD and LCBD-re values presented a significant negative relationship with closeness in all the networks. Moreover, LCBD, LCBD-re and SCBD-prop presented significant positive relationships with pond size, degree and ECELS, respectively, indicating a general trend for all the networks. Meanwhile, LCBD-ad had a significant negative relationship with degree in all the networks. We also found some network dependent relationships when analysing SCBD-prop linked to closeness, for which we obtained both negative and positive relationships.

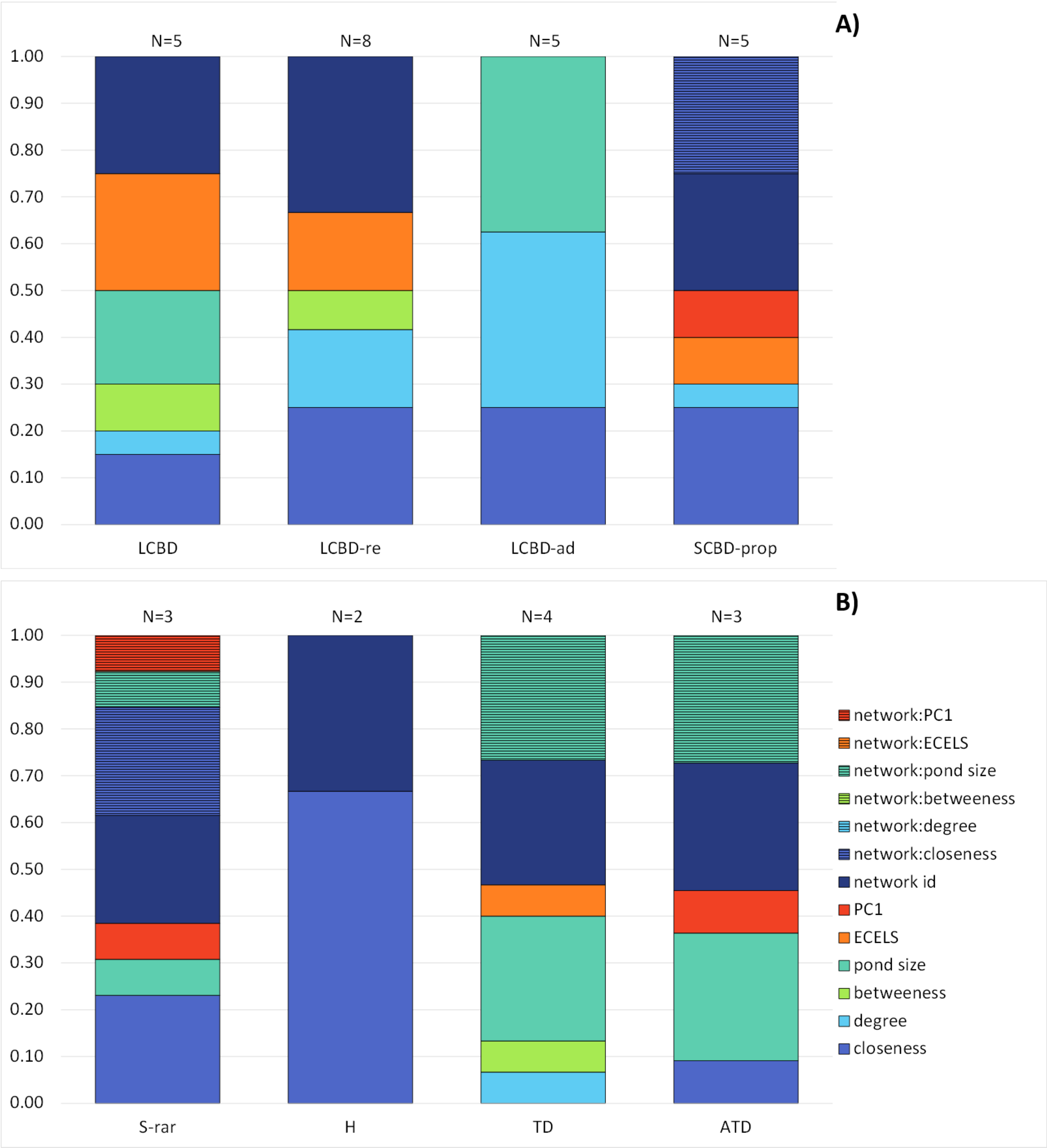

Figure 4.3.3. Barplot showing the relative importance of the different drivers for A) regional biodiversity metrics and B) local biodiversity metrics obtained as the number of models that contain each explanatory variable from the subset with ΔAICc≤2. N indicates the number of models included in the subset.

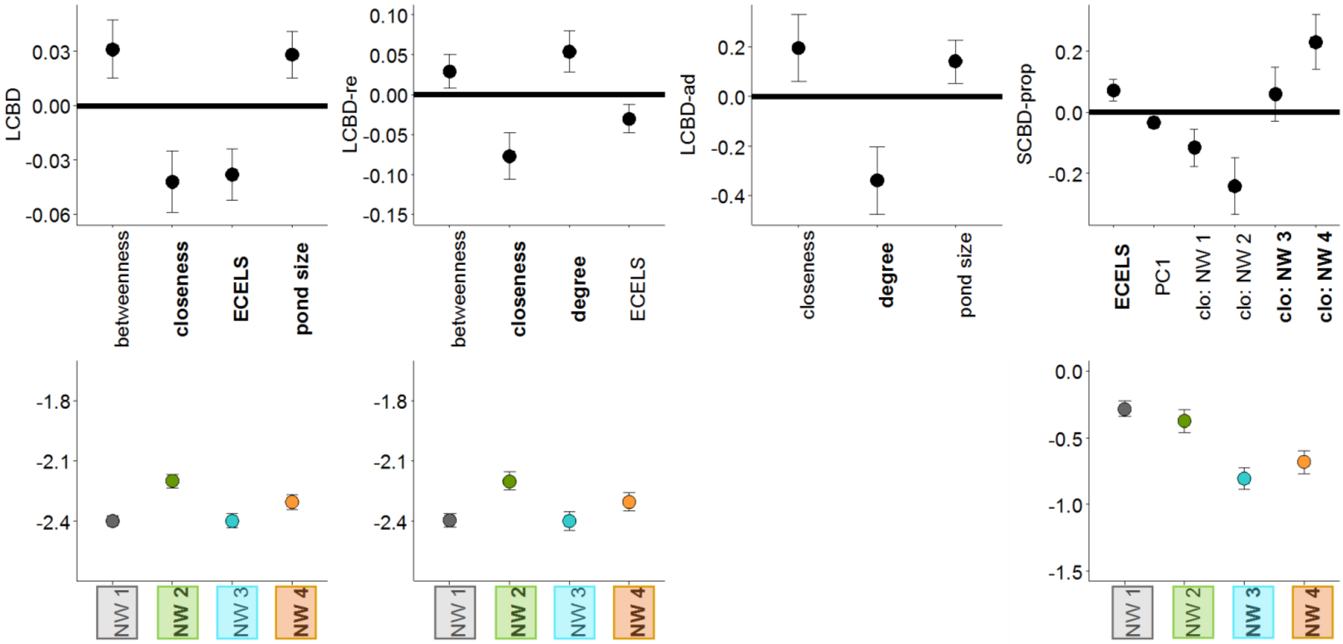

Figure 4.3.4. Beta coefficients (first row) and intercepts (second row) of the different drivers included in the best beta regression model (the one with the highest pseudo-R²) for the regional biodiversity metrics. The explanatory variables with a significant effect are marked in bold. Clo is the abbreviation for closeness.

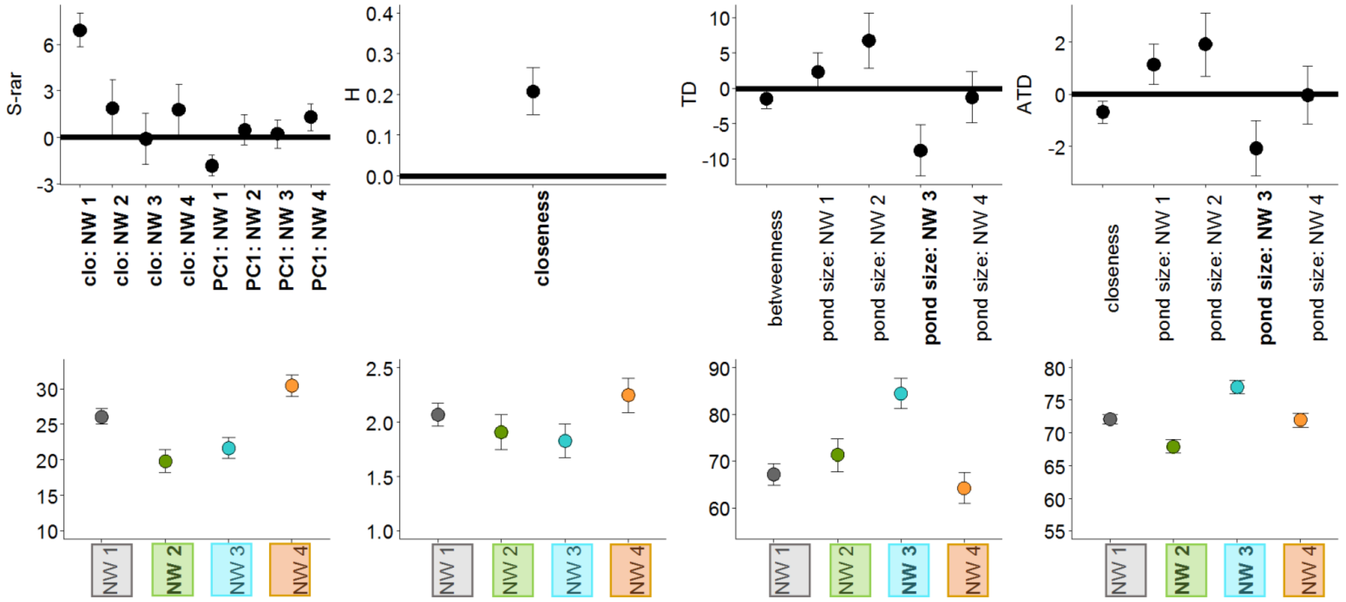

Figure 4.3.5. Slope coefficients (first row) and intercepts (second row) of the different drivers included in the best linear model (the one with the highest adjusted-R²) for the local biodiversity metrics. The explanatory variables with a significant effect are marked in bold. Clo is the abbreviation for closeness.

In the case of the ‘best models’ for each of the local biodiversity metrics, the main drivers were network identity, again indicating intrinsic differences among networks, and closeness. In relation to the role of centrality metrics, closeness presented a positive relationship with alpha diversity (more evident for H, since the relationship with S-rar was network-dependent, ranging from positive to inexistent, but never negative) and negative with one of the phylogenetic diversity metrics (ATD), although non-significant (Figure 4.3.5; SI Table 4.3.3 and 4.3.4). Another negative and non-significant relationship was obtained from the other phylogenetic metric (TD), but this time with betweenness as centrality metric. The role of the variables related to environmental characteristics was network-dependent. In this sense, S-rar had a significant relationship with PC1, which varied from negative to positive, depending on the network. Finally, NW 1 and NW 2 showed a similar positive trend of ATD and TD with pond size, while a negative pattern was obtained in NW 3 and no relationship for NW 4. Consequently, the relationship of pond size with the phylogenetic measures was highly network-dependent. In summary, we detected more general patterns in the local biodiversity metrics with respect to the centrality metrics rather than to environmental characteristics, being in the last case the relationships in most cases network-dependent.

DISCUSSION

Despite differences in the network descriptors (connectance, diameter and linkage density) due to the different arrangements of the patches within each network and different spatial extents of the networks, we found several general patterns in relation to the biodiversity of these systems. The total beta diversity among the ponds that we studied could almost entirely be attributed to species replacement from one pond to another, indicating that dissimilarity among ponds was mainly driven by variation in community composition rather than differences in abundance. This is in accordance with the study of Baselga (2010) who affirms that the replacement component is higher than the abundance difference component in southern Europe and also with studies performed in other lentic and lotic systems (e.g., Hill et al., 2017; Jamoneau et al., 2018; Specziár et al., 2018). Moreover, the dominance of the replacement component over the abundance difference component may reflect a greater influence of environmental variation on community structure rather than of species interactions (Legendre, 2014). Overall, our results pointed out the relevance of the network centrality metrics for the biodiversity values at local and regional scale. However, our results also support the idea that the environmental characteristics of the ponds were also determining the biodiversity of the ponds both at regional and local scale (Hill et al., 2017). Thus, one interesting finding of our study is that most of the biodiversity metrics were not explained by environmental characteristics or network centrality metrics alone but through a combination of them both, consistently with the empirical results of Altermatt et al. (2013).

All the biodiversity metrics calculated (both local and regional ones) presented a significant relationship with at least one centrality metric except the taxonomic indices (TD and ATD). Of the three centrality metrics that we analysed in this study, closeness was more times selected by the models as a significant explanatory variable, followed by degree (which was included only in 2 of the 'best models') and finally, betweenness, which did not have a significant relationship with any of the biodiversity metrics although it was included in some 'best models'. These results highlight that different centrality metrics which measure connectivity at different spatial scales within the network (from a neighboring scale -degree- to a broader scale -closeness-), affect the biodiversity value of the ponds at both regional and local level. However, our results weakly support the expected negative effect of connectivity on the taxonomic distinctness index (Marcantonio et al., 2013; Quiroz-Martínez & Salgado-Maldonado, 2013) since we found a general negative trend but the relationship was not significant. Besides, our results did not support the expected positive effect of connectivity on the average taxonomic distinctness (Brewin et al., 2009) since we observed a negative trend. This means, contrarily to our expectations, that connectivity favored the reduction of the taxonomic breadth of the sites.

When analyzing the effect of centrality focusing in a broad spatial scale (global scale -closeness-) we found, in agreement with other studies, that patches with higher closeness (i.e., that occupy a central position in the network) presented higher species richness and Shannon-Wiener diversity (e.g., Economo & Keitt, 2010; Altermatt et al., 2011; Ai et al., 2013; Carrara et al., 2014). Moreover, closeness was able to explain almost the 30% of the variation observed in Shannon-Wiener diversity among ponds. Furthermore and as we expected, the increase in connectivity (through the increment in closeness) was related to a decrease in the local contribution to beta diversity since a greater centrality of the patches would favor the arrival of individuals by dispersal and thus, the homogenization of the communities (Forbes & Chase, 2002; Schmera et al., 2018). When analyzing the effect of centrality focusing in a smaller spatial scale (neighbouring scale -degree-) we found an expected opposite effect on the two complementary components of the LCBD. Hence, the existence of a greater number of neighboring connections to a pond (ponds with high degree value) generates higher species replacement and lower influence of the abundance differences of individuals in explaining the uniqueness of a pond. Interestingly, degree was only significantly related with the components of LCBD, suggesting an effect of the local position of the node within the network on the biodiversity at regional level. Nevertheless, closeness, that integrates the position of the node in the entire network, was related with biodiversity at both local and regional level. Although we could have expected a similar role of betweenness to the role of terrestrial ‘habitat corridors’, since sites with high betweenness values could be identified as key flow connecters (Economo & Keitt, 2010), we observed weak evidence of its positive relationship with LCBD-re.

In relation to the effect of the environmental characteristics (i.e., water characteristics, pond size and habitat condition), we found more general patterns in the relationships of the regional compared to the local biodiversity metrics. Thus, in the case of the latter, the answers were network-dependent and the habitat condition did not play an important role. When focusing on regional biodiversity metrics, habitat condition has been pointed out as a driver in our study although it presented contradictory effects. On one hand, the ponds presenting the best habitat condition, i.e., the ones with ECELS values close to 100, contributed less to the beta diversity but at the same time were the ones with higher values of SCBD-prop, indicating that they harbour a higher percentage of the species most importantly contributing to the total beta diversity. We conclude from this that the sites with the “mean community”, i.e., sites that harbour the general species abundance pattern and therefore that have a low value of LCBD (Legendre, 2014), are also the ones with the best habitat condition. In this sense, it is important to keep in mind that all the networks that we studied are included in protected natural areas, in which the habitat condition of the ponds is usually high (having ECELS values > 70 that corresponds to ’good’ and ‘high’ habitat conditions; Sala et al., 2004). So, in our study, and in line with the author’s comment on LCBD values (“sites having high LCBD values are not automatically “special” in a good sense, e.g. by harbouring rare species or by being exceptionally rich. Any departure from the overall species abundance pattern increases the LCBD value”; Borcard et al. (2018)), the uniqueness of our ponds would correspond to impoverished sites, since in the studied networks the norm is to be in good or high habitat condition with a “mean” community. Thus, the relationship that we observed between SCBD-prop and the habitat condition is similar to that found in the study by Heino & Grönroos (2017) in which some niche characteristics were strongly related to SCBD. In our study, the water characteristics favored in some cases or were detrimental in others to species richness depending on the network indicating that some species can be either favored by certain water variables as it was also previously reported for total nitrogen (Tolonen et al., 2005) or not favored by, for instance, pH and chlorophyll *a* concentration (Jeffries, 1991; Brodersen et al., 1998). We did not find, however, a relationship between the water characteristics and both beta diversity and taxonomic distinctness as García-Girón et al. (2019) did find. In relation to the effect of habitat size, we found that larger ponds presented higher uniqueness indicating that larger habitats can host a greater number of unusual species combinations (Legendre & De Cáceres, 2013), which may be attributed to the probability of presenting a greater habitat heterogeneity (MacArthur & Wilson, 1967; Rosenzweig, 1995). Moreover, we found a network-dependent relationship between habitat size and both taxonomic distinctness indices (positive or negative). This result suggests that in some of the networks studied, the increase in pond size favors a greater taxonomic breadth, whereas in others, on the contrary, it was not favored. Likewise, network identity became highly significant as a main factor, indicating that for both local and regional biodiversity patterns, the intrinsic differences among networks are important.

In summary, our results indicate the presence of drivers of biodiversity operating at different network scales within the metacommunities. In previous studies performed in different habitats (Tonkin et al., 2016b; da Silva et al., 2018) authors found out that both environmental (local) variables and spatial variables were responsible of a higher contribution of sites to beta diversity. Thus, our results for biodiversity at regional level are in the same line. Moreover, the network centrality metrics that we used resulted to be complementary among them since they presented relationships with different biodiversity metrics (both local and regional), their effects were in some cases opposite (positive or negative) and their effects were in most cases general for the different networks that we studied. On the other hand, our results emphasize the importance of using several different biodiversity metrics in the same study since this enables us to analyze complementary information about the biodiversity value (Gascón et al., 2009; Heino et al., 2005; Gallardo et al., 2011). Therefore, our work contributes to improve the knowledge of the relationships established between the components of the beta diversity and the environmental and spatial properties since this has been a recent question addressed and it is still unclear (Hill et al., 2017; Specziár et al., 2018). Indeed, as far as we are concerned, this is the first empirical study that using the graph theory relates network centrality metrics calculated from the graph with the recent approach to partition total beta diversity into sites and species contributions since, for the moment, previous studies only analysed local biodiversity metrics such as species richness and ignored regional biodiversity.

5. GENERAL DISCUSSION

5.1. Biodiversity and the importance of scale: from pond size to pond network

Biodiversity is a multidimensional concept encompassing many scales of variation that was originally partitioned into three components: alpha, beta and gamma (Whittaker, 1960). Within biogeography, there is evidence for variation in the spatial processes and patterns driving biodiversity at different spatial scales (Rosenzweig, 1995; Whittaker et al., 2001). Although Barton et al. (2013) stated that the patterns and processes shaping the spatial scaling of beta diversity were not thoroughly explored, from then on, many studies detected the effect of spatial scale on beta diversity (Alahuhta & Heino, 2013; Fu et al., 2019; Leboucher et al., 2019; Pitacco et al., 2019). In the case of alpha diversity (i.e. species richness), its dependence on spatial scale is also demonstrated (Rahbek, 2005; Whittaker et al., 2001; Chase et al., 2019). However, it remains poorly understood how species sorting and dispersal processes interact to influence community variation across scales since their relative contributions are clearly dependent on the spatial context in which they are measured (Leboucher et al., 2019). Therefore, to better account for scale effects on biodiversity, in this thesis we worked with a gradient of scales: from a range of different pond sizes to a range of pond networks with different spatial extents, considering its effects not only at local level but also including a metacommunity perspective.

The idea that habitat size is highly determinant for biodiversity is well stablished in the scientific community (MacArthur & Wilson, 1963, 1967). Nevertheless, and in accordance with other studies (García-Valdecasas et al., 1984; Friday, 1987; Jeffries, 1991; Bilton et al., 2001a), we did not find the expected positive relationship between pond size and one of the most usually used biodiversity metrics at community level (i.e. species richness), neither when considering a small spatial extent (Chapter 1) nor when comparing different pond networks with different spatial extents (Chapter 3). However, we did find some relationships with other biodiversity metrics measured at local level but linked to phylogenetic relatedness of the taxa (such as the taxonomic distinctness and the average taxonomic distinctness), although these relationships were pond network dependent (Chapter 3).

On the other hand, our results showed a consistent (so, not pond network dependent) and positive trend (Chapter 3) between pond size and the local contribution to the beta diversity (i.e. LCBD; which indicates the uniqueness of the biological community at each site), meaning that larger ponds presented a higher contribution to beta diversity (i.e., more "unique" communities when compared to smaller ponds). The fact that the larger ponds studied were also the ponds with highest uniqueness in community composition, could be due to having a higher habitat heterogeneity (Krauss et al., 2004) and thus, potential niches that would shelter more unique assemblages (Heino, 2000). In fact, habitat size has already been identified as a key factor determining communities composition (e.g., Heino, 2000; Rundle et al., 2002; Semlitsch et al., 2015), and we also found evidences in this sense (Chapter 1). Accordingly, our results indicate that pond size influence community composition through the variation of the abundances of certain species, suggesting a differential response to pond size depending on taxa. Moreover, dispersal ability may also potentially account for regional variation in community structure, helping or preventing the organisms from reaching or not more suitable habitats (Padial et al., 2014; Heino et al., 2017). When deeply analysed this possibility, we found that pond size was determinant for the composition of active dispersers at smaller spatial extents (smallest pond network), whereas at larger spatial extents (larger pond network), pond size was determinant for passive dispersers, both plants and macroinvertebrates (Chapter 2).

When focusing at network level, the organisms may find some kind of dispersal limitation linked to the spatial extent of the network (Heino, 2013b). However, dispersal in freshwater taxa is difficult to study directly and some dispersal events may remain undetected (Bilton et al., 2001b; Pinceel et al., 2016 but see Vanschoenwinkel et al., 2009). Therefore, an indirect approach that classifies the organisms according to its dispersal mode, is normally used to take into account different dispersal behaviours (Bilton et al., 2001b). In this sense, organisms which are passively dispersed, depend on external vectors (i.e. other organisms or wind dispersal, etc.) and therefore this limits their ability to select suitable habitats and increases the influence of spatial processes (Vanschoenwinkel et al., 2008; De Bie et al., 2012; Heino et al., 2014) whereas organisms that actively disperse can, by self-generated movement, reach new habitats to colonize (Rundle et al., 2007). Therefore, different organisms may respond differently to processes at different scales (Steinitz et al., 2006; Soininen et al., 2007b; Logue et al., 2011; Maloney & Munguia, 2011; Saito et al., 2015). In line with this, when we tested the effects of geographic distance on groups with different dispersal modes, the trend obtained was not the same for all of them (Chapter 2). Active dispersers from the smallest pond network showed a decay in similarity whereas neither passive dispersers nor plants did, in agreement with the idea that the same landscape could be perceived differently depending on the taxa (Borthagaray et al., 2014).

Our results (Chapter 3) also support the idea that the spatial extent is of relevance when analysing biodiversity patterns (Whittaker et al., 2001; Barton et al., 2013; Chase & Knight, 2013; Chase et al., 2019). However, when analysing the patterns observed on local and regional biodiversity values, a different response was observed. Thus, the patterns observed for the regional biodiversity values were usually more context independent, indicating consistent patterns among the 4 networks studied with different spatial structures (i.e. including differences on their extent), suggesting consistency in the responses of the regional biodiversity (i.e. regardless of the network structure) to both environmental and connectivity characteristics. In this line, Heino et al. (2015a) neither observed a relationship between beta diversity and spatial extent, contrarily to the patterns of various terrestrial taxa that usually present that relationship. Moreover, the total beta diversity of the 4 networks (the unique value obtained for the whole network) was mostly explained by the replacement component, with the abundance difference component being much lower. This fact could be explained by the small extent of all the networks analysed despite the existent range of spatial extents, since, as it has been proposed, the replacement component is the dominant driver of beta diversity at smaller extents (Antão et al., 2019). In contrast, when analysing the patterns of local biodiversity values, more context dependent relationships arise, indicating a higher network dependence of local biodiversity values (Chapter 3).

5.2. Environmental versus spatial factors

One of the central topics of community ecology is the estimation of the relative roles of environment, space and their interactions in structuring community composition (Soininen, 2014; Vellend, 2016). Thus, examining the relative influence of space and environment on the similarity of species composition among sites (i.e., beta diversity; Whittaker, 1972) may help resolve the underlying processes. For example, the different metacommunity archetypes place different emphasis on the importance of environmental factors (species sorting and mass effects) and spatial factors (patch dynamics and neutral theory) (Cottenie, 2005; Logue et al., 2011; Leibold & Chase, 2018). Moreover, the prevalence of the different metacommunity dynamics is thought to be influenced by the spatial extent of the study area, since increasing the spatial extent results in an increase in habitat heterogeneity and dispersal limitation between the different sites (Cottenie, 2005; Heino et al., 2014; Gianuca et al., 2017). On the other hand, it can be expected that spatial structuring would be stronger for larger spatial extent studies, whereas in smaller ones environmental control would increase (Heino et al., 2012). Therefore, throughout this thesis, the role of different environmental and spatial factors in community assemblages was analysed using, not only community composition information (Chapter 1 and Chapter 2), but also regional biodiversity metrics (Chapter 3). Our results showed that, in the smallest extent network, neither there was an effect of any of the measured environmental factors (water trophic state and habitat structure) nor of the spatial ones (connectivity) on community structure or composition (Chapter 1), with the exception of pond size that affects community composition. However, when considering a wider range of networks with different spatial extents (Chapter 2 and Chapter 3), some significant results were obtained. Interestingly, when considering species composition (Chapter 2), the importance of environmental factors was more prevalent than that of spatial ones. Contrarily to what could be expected according to Heino et al. (2012), we did not find a stronger environmental control in the smaller extent in comparison with the larger extent. On the contrary, environmental control was more evident in the larger network, probably as a consequence of a higher dispersal limitation that prevents mass effects (Heino et al., 2014). Hence, our results from that study suggest that, as it was also detected in other metacommunities, species composition varied mostly due to environmental conditions between sites rather than spatial effects (Cottenie, 2005; Heino & Soininen, 2010; Heino et al., 2015a). However, active dispersers' assemblages showed a relationship with space, since a spatial distance-decay of community similarity was observed (Chapter 2). Overall, the results are congruent with previous studies also in ponds (Cottenie, 2005; Ng et al., 2009; Vanschoenwinkel et al., 2007; García-Girón et al., 2019) which suggested that a combination of mass effects, dispersal limitation, and species sorting may be determining pond community structure. On the other hand, it has been stated that in observational studies it is difficult to discern if simultaneous metacommunity mechanisms are operating (Logue et al., 2011) since we might be observing the net effect of all metacommunity processes that are taking place (e.g. Spasojevic et al., 2014). Nevertheless, we did find that spatial distance was of relevance when trying to identify the metacommunity archetype dominating the assembly of communities in a certain network (Chapter 2). Indeed, it has been hypothesised (Cottenie, 2005; Soininen, 2014) that increasing the spatial extent of a study would favour the species sorting over the mass effects. Our results support this hypothesis but only for active dispersers, since passive dispersers showed always a response that better fits under the species sorting dynamics. Other studies also detected a prevalence of species sorting at small spatial scales (Cottenie, 2005; Soininen, 2014), concluding that species sorting could also prevail at smaller spatial scales for organisms with lower dispersal efficiency, which might be the case in our study for faunal passive dispersers.

Finally, our findings (Chapter 3) pointed out the role of both spatial and environmental factors on the biodiversity patterns from the metacommunities we were focused on. Environmental factors alone did not independently explain biodiversity patterns but we found support for a combination of environmental and spatial factors and, conversely, some other biodiversity patterns were explained by spatial factors alone. Therefore, spatial factors showed a more predominant role than environmental factors as biodiversity drivers. Hence, our results for the beta diversity are in agreement with other studies on beta diversity patterns (Tonkin et al., 2016b; Hill et al., 2017b; da Silva et al., 2018), that stated that beta diversity metrics are governed by both environmental and spatial factors. Summarising, mainly spatial factors and to a lesser extent, environmental factors, played an important role for the biodiversity patterns that we found at both regional and local level.

5.3. Connectivity and pond network structure

Appealing to the island biogeography theory, species richness should be negatively correlated with the degree of isolation of the habitat (MacArthur & Wilson, 1963, 1967; Scheffer et al., 2006; Kadmon & Allouche, 2007; Fahrig, 2013). Within landscape ecology, patch isolation depends on the amount of habitat within some distance of the patch (Fahrig, 2013). In this sense, many studies have found negative effects of patch isolation on species richness (e.g., Bailey et al., 2010; Schüepp et al., 2011) although to a lesser extent other works have found an increase of species richness favoured by isolation (e.g., Scheffer et al., 2006). Conversely, connectivity has been considered a crucial factor for maintaining biodiversity at metacommunity level (Loreau et al., 2003a; Matisziw & Murray, 2009; Staddon et al., 2010). Moreover, it is well-known the homogenising effect on species composition of connectivity at a regional level since it can decrease community dissimilarity among the patches, i.e., decreasing beta diversity (Chase, 2003; Mouquet & Loreau, 2003; Borthagaray et al., 2015b). Additionally, isolation-centrality (i.e., connectivity) measures are also of relevance since they are normally used as an indirect method to assess dispersal at metacommunity level (Jacobson & Peres-Neto, 2010). We observed a clear role of connectivity in structuring the metacommunities in networks differing in spatial extent (Chapter 1, 2 and 3). In fact, connectivity is highly relevant since it can modulate the effect of spatial extent, for example making that even in the larger networks dispersal was not limited due to the great number of connections among the patches (Chapter 3). In the smallest network that we studied, the effect of connectivity was thought to be responsible for both the homogenization along the pond network of the community structure and composition, and the absence of a species-area relationship (Chapter 1). Thus, in agreement with the theoretical predictions (Chase, 2003; Mouquet & Loreau, 2003; Borthagaray et al., 2015b), connectivity seemed to have a homogenising effect on the community composition of the pond networks (Chapter 1, 2 and 3). Distance can be considered an important factor for connectivity since a certain distance can prevent the connectivity among the patches and thus, could be limiting for the dispersal of organisms (e.g., Heino et al., 2014). When connectivity was not explicitly calculated but instead it was measured the spatial distance between the two furthest ponds that encompassed the network (Chapter 2), we found that neither in the smallest nor in a larger network, spatial distance was limiting dispersal since geographical distance-decay was not observed with the exception of the communities of active dispersers in the smaller network. Therefore, connectivity might be favoured and dispersal might be not limited in these metacommunities since spatial distances between ponds were small enough not to prevent dispersal among ponds (Heino et al., 2014). Moreover, our results showed a relationship between the centrality metrics (and therefore, connectivity) and biodiversity (Chapter 3). As it was expected, connectivity was positively related with alpha diversity. In addition, in two of the four networks analysed, connectivity was positively related with a higher proportion of species contributing to beta diversity (SCBD-prop). On the other hand, as it was hypothesised, we found, overall, a negative relationship between connectivity and the components of beta diversity. However, sites with a greater number of connections with the rest of the patches of the network, were also sites contributing more in the replacement of species among ponds suggesting that dispersal was favoured in those patches (Henriques-Silva et al., 2013; Gianuca et al., 2017). These findings agree with other studies (e.g., da Silva et al., 2018; Landeiro et al., 2018) that also found a relationship between the components of the beta diversity and spatial structures. Summarising, we have observed that in networks of Mediterranean temporary ponds, the situation (central-isolated) of a pond with respect to the rest of the network is decisive for biodiversity at both local and regional level.

6. CONCLUSIONS

In relation to the first objective of this thesis (*to test the effect of pond size on the composition and structure of the macrofaunal community from a network of temporary ponds and to analyse if other environmental factors could modulate the effects of pond size on the community*), we conclude that:

- In the smallest pond network, there is an unclear relationship between pond size and macrofauna, since we found a significant effect on community composition but did not find an effect on community structure parameters such as richness, taxonomic diversity or body size diversity. So, the abundance of some taxa, such as *Gyraulus laevis* (Planorbidae), *Piona* sp. (Pionidae) and *Aeshna mixta* (Aeshnidae), increased in larger ponds, whereas the abundance of other taxa, such as *Agabus* sp. (Dytiscidae), *Chaoborus flavicans* (Chaoboridae) and *Culex theileri* (Culicidae), had their maximum density in the smaller ones (Chapter 1).

- The effect of pond size detected in the faunal composition is not due to a covariation between pond size and connectivity, trophic state or macrophytes biomass (Chapter 1).

- In all the networks studied, larger ponds present also higher "unique" communities (which does not mean that they are "special" in a good sense). On the other hand, only in two of the networks, the increase in pond size favors a greater taxonomic breadth of the communities (Chapter 3).

In relation to the second objective of this thesis (*to test the relative role of environmental versus spatial factors on generating differences on community assemblages*), we conclude that:

- Overall, we found that environmental distance had larger effects than geographical distances in networks of temporary ponds and for the different organisms' dispersal groups: macrofaunal active and passive dispersers, and plants (Chapter 2).

- Our results indicate the presence of drivers of biodiversity operating at different network scales within the metacommunities of the 4 pond networks studied. Both environmental factors (water characteristics, pond size and habitat condition) and connectivity are drivers of biodiversity at regional and local level. However, most of the biodiversity metrics were not explained by environmental characteristics or network metrics (i.e., connectivity) alone, but through a combination of them both (Chapter 3).

In relation to the third objective of this thesis (*to propose a new framework linking distance-decay patterns to different metacommunity dynamics taking into account both the dispersal ability of the organisms and the spatial extent of the network*), we conclude that:

- We detected the existence of differences in the distance-decay patterns among taxa groups depending on their dispersal strategies and the spatial extent of the pond network. Thus, in the larger pond network, we detected an environmental distance-decay in the three biotic groups, whereas no spatial distance-decay for any of the groups. In the smaller pond network, both macrofaunal active and passive dispersers presented a decay with environmental distance, plants did not suffer a decay in similarity, and only macrofaunal active dispersers had a decay with geographical distance (Chapter 2).

- The three groups of organisms analysed (macrofaunal active and passive dispersers, and plants) seemed to follow the species sorting archetype despite having different dispersal modes, whereas, in the smaller pond network, each group better fitted a different archetype. In the latter case, the macrofaunal active dispersers better fit the trend expected when the mass effects dominates over the rest of archetypes; the macrofaunal passive dispersers showed a pattern that was expected when species sorting prevails and, in the case of the plants, the pattern was difficult to associate with any metacommunity archetype. This highlights that the type of metacommunity archetypes ruling community similarities is strongly influenced by spatial extent (Chapter 2).

In relation to the fourth objective of this thesis (*to examine how the network structure and the connectivity within the network determines patterns of biodiversity in metacommunities taking also into account the potential role of local factors such as habitat area and quality, and environmental characteristics*), we conclude that:

- Closeness centrality, which is a network metric that integrates the broader spatial scale, is related with biodiversity at both local and regional level. Patches with higher closeness (i.e., that occupy a central position in the network) present higher alpha diversity (i.e., both species richness and Shannon-Wiener diversity). Moreover, the increment in closeness is related to a decrease in the local contribution to beta diversity since a greater centrality of the patches would favor the arrival of individuals by dispersal and thus, the homogenization of the communities. Degree centrality, which is a network metric that integrates a neighbor scale, is only related with the components of the local contribution to beta diversity, suggesting an effect of the local position of the node within the network on the biodiversity at regional level (Chapter 3).

- The total beta diversity among the ponds that we studied in the four pond networks could almost entirely be attributed to species replacement from one pond to another, indicating that dissimilarity among ponds is mainly driven by variation in community composition rather than differences in abundance. On the other hand, all the ponds studied present a good habitat condition making that the ponds with the best conditions are, at the same time, the ones contributing less to the beta diversity but harboring a higher percentage of the species most importantly contributing to the total beta diversity (Chapter 3).

- Our results weakly support the expected negative effect of connectivity on the taxonomic distinctness index. Besides, our results do not support the expected positive effect of connectivity on the average taxonomic distinctness, since we observe a negative trend. Thus, contrarily to our expectations, connectivity favors the reduction of the taxonomic breadth of the communities (Chapter 3).

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APPENDIX 1

(Supporting information of Chapter 1)

Biodiversity of the aquatic macrofauna of Vila Nova de Milfontes temporary ponds, with the first citation of *Cyphon hilaris* Nyholm, 1944 (Coleoptera: Scirtidae) in Portugal

Abstract: The aquatic community of 12 temporary ponds located in Vila Nova de Milfontes (SW Portugal) has been sampled in April 2013. We identified a total of 78 taxa, where insects (59 taxa), and within them coleopterans (29 taxa), were the best represented groups. It should be noted the presence of six amphibian species, four exotic species (2 gastropods, 1 heteropteran and 1 coleopteran) and the first record of *Cyphon hilaris* Nyholm, 1944 in Portugal.

Key words: Scirtidae, macroinvertebrates, amphibians, checklist, Mediterranean wetlands, Portugal.

Temporary ponds are, in general, small and shallow water bodies, easily overlooked and vulnerable to a large number of human activities (Cancela da Fonseca et al., 2008). They are a biologically important habitat type, renowned both for their specialised assemblages and the considerable numbers of rare and endemic species they support (Giudicelli & Thiéry, 1998; Quézel, 1998; Williams et al., 2001; Zacharias & Zamparas, 2010). Mediterranean Temporary Ponds are protected under the Habitats Directive 92/43/CEE, although management plans concerning these special and fragile habitats are still rare, particularly because the state of knowledge is still insufficient to establish management procedures (Grillas & Roché, 1997; Cancela da Fonseca et al., 2008). Nevertheless, some LIFE projects have already been carried out in some Mediterranean regions like Provence and Corsica in France, or Minorca and Valencia in Spain (Grillas et al., 2004; Fraga et al., 2010; Sancho & Lacomba, 2010).

The SW of Portugal is a key area for temporary ponds, with a high density of these habitats. These ponds are important due to the existence of some vulnerable species of flora and fauna (e.g., *Juncus emmanuelis* A. Fern. & J.G. García, *Hyacinthoides vicentina* (Hoffmanns. & Link) Rothm., *Dussartius baeticus* (Dussart, 1967), *Acilius duvergeri* Dettner, 1982; Bergsten & Miller, 2006; Canha & Pinto-Cruz, 2010), and they are considered to have a high conservation value for aquatic assemblages such as plants, coleopterans or amphibians (Ribera, 2000; Beja & Alcazar, 2003; Pinto-Cruz et al., 2009). In addition, the amphibian species are included in conservation international agreements, such as the Convention on the Conservation of European Wildlife and Natural Habitats (known as Berne Convention). Although a large number of these temporary ponds are included in the Southwest Alentejo and Vicentine Coast Natural Park (PNSACV), the degradation and loss of these habitats is a matter of great concern (Canha & Pinto-Cruz, 2010). It is important to note that some of these habitats have been included in a conservation European project that will be developed during the next years: "LIFE Charcos. Conservation of Temporary Ponds in the Southwest Coast of Portugal" (LIFE12 NAT/PT/000997). Due to its high conservation value, many studies on flora (Pinto-Cruz et al., 2009; 2011) and fauna have already been conducted in this area. Regarding the fauna, different groups of the aquatic community of the ponds have previously been studied. These works were mainly focused on amphibians (Beja & Alcazar, 2003), aquatic insects (Chaves, 1999), copepods and cladocerans (Caramujo and Boavida, 2010), large branchiopods (Machado et al., 1999) and ostracods (Martins et al., 2010). Nevertheless, we have not found a study of all the aquatic macrofauna community of this site. Thus, this work aims to present the first description of the aquatic macrofauna communities of these Mediterranean temporary ponds.

The survey was carried out between 22nd and 23rd April 2013 in twelve temporary ponds located near the village of Vila Nova de Milfontes (37°45' N 8°48' W), within the Southwest Alentejo and Vicentine Coast Natural Park in Portugal. They are situated on a coastal sandy plateau protected by consolidated dunes on the west, and by a wooded area to the east (Caramujo and Boavida, 2010). This is a set of ponds of different sizes, shapes and depths which in turn, show variations of these features individually throughout the year (Chaves, 1999). The climate is Mediterranean with oceanic influence. The soil is highly permeable and ponds fill mainly with rain water (Caramujo and Boavida, 2010). Each pond was sampled only once. Macroinvertebrate samples were taken using a dip net with a diameter of 22 cm and a mesh size of 250 µm. The sampling procedure was based on 20 dip-net sweeps in rapid sequence spanning all of the different mesohabitats. Samples were preserved *in situ* in ethanol 70°. Individuals were identified to species level whenever possible, except in the case of chironomids, which were identified to subfamily.

The macrofauna found in the studied area included 78 taxa, 59 of which were insects. Within the insects, Coleoptera, Diptera and Heteroptera were the best represented orders with 29, 12 and 11 taxa, respectively. However, we should take into account that Diptera richness was underestimated, because non-bitting midges were identified to a low taxonomic resolution (Chironomidae is one of the richest families in temporary Mediterranean ponds; Bazzanti et al., 1997; Boix et al., 2001). Odonata (five taxa) and Ephemeroptera (two taxa) were the insect orders less represented (Appendix 1 Table 1). It should also be highlighted the presence of six different species of amphibians despite the global (Houlahan et al., 2000) and local decline in its populations especially due to agricultural intensification (Beja & Alcazar, 2003). Each pond had a minimum of three amphibian species.

The aquatic insects of these ponds were previously studied by Chaves (1999) in a larger study both in time and number of samples. The mentioned study was focused on Coleoptera, in which 9 families and 30 genera were found, whereas in the present study we recorded 12 families and 28 genera. Taking into account only adult coleopterans, because they can usually be identified to species level, Chaves (1999) found 39 species while we only found 17. Although the ponds were sampled only in one occasion, it is interesting to note that some taxa were not recorded before, such as *Gyrinus caspius* Ménétries, 1832, *Gyrinus dejeani* Brullé, 1832, *Ilybius* sp., *Porhydrus* sp., *Liopterus atriceps* (Sharp, 1882), *Cybister tripunctatus africanus* Laporte, 1834, *Helophorus* cf. *lapponicus* Thomson, 1853, *Hydraena (Hydraena)* sp., *Cyphon hilaris* Nyholm, 1944 and *Stenopelmus rufinasus* Gyllenhal, 1835. In addition, we found some soil indeterminate coleopterans of the subfamily Alleculinae that have also been found in other temporary ponds in northeastern Iberian Peninsula such as Espolla pond (Boix et al., 2000) and Albera temporary ponds (unpublished data).

The most remarkable finding was the presence of the coleopteran species *Cyphon hilaris* Nyholm, 1944, as it has not been cited before in Portugal. *C. hilaris* is mainly distributed along the Baltic and Atlantic coasts of Europe, from Finland to Spain (Hannappel & Paulus, 1997; Cuppen & Foster, 2005; Klausnitzer, 2009). In the case of the Iberian Peninsula, it has been previously found only twice, in Galicia and Doñana (Millán et al., 2005; Klausnitzer, 2009). It is common to find this species in acidic and mesotrophic environments, although it is not rare in reedbeds (Cuppen & Foster, 2005; Klausnitzer, 2009). The Scirtidae is one of the least known families of aquatic coleopterans in the Iberian Peninsula, so the citations of this family in the region are particularly interesting due to the general lack of data (Ribera et al., 2009-10). In the Iberian Peninsula, the genus *Cyphon* Paykull, 1799 includes around 13 species, with two of them, *C. padi* (Linnaeus, 1758) and *C. coarctatus* Paykull 1799, with unconfirmed presence (they are not recorded by Klausnitzer (2009), although they appear in the checklist by Ribera et al. (1998)).

It is also interesting to note the presence of several exotic species in these ponds. As it is already well-known, invasive non-indigenous species are one of the main threats to biodiversity (Mack et al., 2000; Clavero & García-Berthou, 2005) and temporary ponds are not exempt of suffering this pressure (e.g., Gutiérrez-Yurrita et al., 1998; Margaritora et al., 2001), although in some cases the abiotic factors can attenuate the effects of the invasive species in these environments (Adams, 2000; Gerhardt & Collinge, 2007). In the present study we found 4 non-indigenous species: *Physella acuta* Draparnaud, 1805, *Ferrissia* sp., *Trichocorixa verticalis* (Fieber, 1851) and *Stenopelmus rufinasus* Gyllenhal, 1835. Both *P. acuta* and *Ferrissia* sp. are gastropods from North America that have long been regarded as indigenous to continental Europe (García-Berthou et al., 2007; Marrone et al., 2011). In the case of the heteropteran *T. verticalis*, recent studies seem to confirm its establishment in Europe (Günther, 2004; Sala & Boix, 2005) and its continuing expansion (Rodríguez-Pérez et al., 2009; L'Mohdi et al., 2010; Carbonell et al., 2012). This citation from Vila Nova de Milfontes represents an intermediate population between the known localities from the south (Doñana, Algarve; Millán et al., 2005; Sala & Boix, 2005) and the northern locality of Santarém (Kment, 2006). In the case of the coleopteran *S. rufinasus*, which has recently been cited from Portugal for the first time (Carrapiço et al., 2011), it has probably been introduced in Europe with the aquatic fern *Azolla filiculoides* Lamarck, from which it feeds. Janson (1921) suggested that immature stages of the beetle could be carried with its host plant attached to waterfowl, which can act as a dispersal vector. However, the association of the aquatic fern with the rice fields, together with its use as a green manure suggests that humans probably also have a role in its dispersal (Lumpkin & Plucknett, 1985; García-Murillo et al., 2007). In fact, in the other Iberian places where *S. rufinasus* has been located, *A. filiculoides* is also present (Fernández et al., 2005; Mor et al., 2010). Despite not having found *A. filiculoides* in our study site, it has been cited in the nearby region of Odemira (Canha & Pinto-Cruz, 2010), although some authors collected this species also from *Lemna* sp. (Carrapiço et al., 2011).

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Appendix 1 Table 1. Checklist of the aquatic macrofauna found in this study from Vila Nova de Milfontes (Portugal) with the pond codes and geographical coordinates. **B16** (37°45’34.8”N 8°47’39.1”W), **B23** (37°44’54.0”N 8°47”47.1”W), **B18** (37°45’14.6”N 8°47’33.8”W), **B9** (37°45’15.6”N 8°47’25.3”W), **B22** (37°44’53.5”N 8°47’53.3”W), **B14** (37°45’31.5”N 8°47’32.0”W), **B27** (37°45’21.0”N 8°47’54.5”W), **B26** (37°45’01.1”N 8°47’49.5”W), **B20** (37°45’02.6”N 8°47’40.8”W), **B12** (37°45’28.2”N 8°47’27.5”W), **B20bis** (37°44’54.0”N 8°47’34.7”W), **B17** (37°45’21.5”N 8°47’32.1”W).

	B16	B23	B18	B9	B22	B14	B27	B26	B20	B12	B20b	B17
Ph. Cnidaria												
Cl. Hydrozoa												
O. Anthomedusae												
F. Hydridae												
Hydra sp.	-	-	-	-	-	-	-	-	+	-	-	-
Ph. Platyhelminthes												
Cl. Turbellaria												
O. Neorhabdocoela												
Neorhabdocoela indet.	-	-	+	-	-	-	+	+	+	+	+	+
O. Tricladida												
F. Dugesiidae												
Dugesiidae indet.	-	-	-	+	-	-	+	-	+	+	-	+
Ph. Annelida												
Cl. Hirudinea												
O. Arhynchobdellida												
F. Erpobdellidae												
Dina lineata (OF Müller, 1774)	-	-	-	-	-	-	+	-	-	-	-	-
Cl. Oligochaeta												
O. Opisthopora												
F. Lumbricidae												
Eiseniella tetraedra (Savigny, 1826)	-	-	-	-	+	-	-	-	-	-	-	-
O. Tubificida												
F. Enchytraeidae												
Enchytraeidae indet.	+	+	-	+	-	+	+	+	+	+	+	+
F. Tubificidae												
Tubificidae indet.	-	-	+	-	-	-	-	-	-	-	-	-
Ph. Arthropoda												
Cl. Arachnida												
O. Prostigmata												
F. Pionidae												
Piona sp.	-	-	-	+	+	-	+	-	+	-	-	+
Tiphynae indet.	+	-	-	+	-	+	+	-	-	+	-	+
Cl. Insecta												
O. Odonata												
F. Aeshnidae												
Aeshna mixta Latreille, 1805	-	-	-	-	+	-	+	-	+	-	-	-
F. Coenagrionidae												
Coenagrionidae indet.	+	-	-	-	-	-	-	-	-	-	-	-
F. Lestidae												
Lestes sp.	-	-	-	+	-	-	-	-	-	-	-	-
F. Libellulidae												
Sympetrum fonscolombii (Sélys, 1840)	-	+	+	+	+	-	+	+	+	+	+	+
Sympetrum striolatum (Charpentier, 1840)	+	-	-	+	-	-	+	-	-	+	+	+
O. Ephemeroptera												
F. Baetidae												
Cloeon gr. *dipterum*	+	-	-	+	+	+	+	+	+	+	-	+
Cloeon schoenemundi Bengtsson, 1936	-	+	-	-	-	-	-	-	-	-	-	-

	B16	B23	B18	B9	B22	B14	B27	B26	B20	B12	B20b	B17
O. Heteroptera												
F. Corixidae												
Corixa afinis Leach, 1817	-	-	-	+	-	+	+	+	-	-	-	-
Hesperocorixa linnaei (Fieber, 1848)	-	-	-	-	-	-	-	-	+	-	-	-
Sigara lateralis (Leach, 1817)	-	-	-	-	-	-	-	-	-	-	-	+
Sigara nigrolineata (Fieber, 1848)	-	+	-	-	-	-	-	-	-	-	-	-
Sigara cf. *scotti* (Douglas & Scott, 1868)	-	-	-	-	-	-	-	-	-	+	-	-
Trichocorixa verticalis verticalis (Fieber, 1851)	-	-	-	-	-	+	-	-	-	-	-	-
F. Gerridae												
Gerris thoracicus Schummel, 1832	+	+	-	+	+	+	+	+	-	+	+	+
F. Notonectidae												
Anisops sardeus Herrich-Schäffer, 1849	+	-	+	+	+	+	+	-	-	+	-	+
Notonecta glauca Linnaeus, 1758	-	+	-	-	-	+	-	-	-	-	-	-
Notonecta meridionalis Poisson, 1926	+	+	+	+	+	+	-	+	+	-	+	-
F. Pleidae												
Plea minutissima Leach, 1817	-	-	-	+	-	+	+	-	-	-	-	-
O. Coleoptera												
F. Dryopidae												
Dryops doderoi Bollow, 1936	-	-	-	-	-	-	+	-	-	-	-	-
F. Dytiscidae												
Agabus sp. (larva)	+	-	+	-	-	+	-	-	-	-	+	+
Bidessus goudoti (Laporte, 1834)	-	+	-	+	-	-	-	+	-	+	+	+
Cybister tripunctatus africanus Laporte, 1834	-	-	-	-	-	-	+	-	-	-	-	-
Graptodytes flavipes (Olivier, 1795)	+	-	+	+	-	+	-	-	-	+	-	-
Hydroporus tessellatus (Drapiez, 1819)	+	-	+	+	-	-	-	+	+	+	-	-
Hydrovatus cuspidatus Kunze, 1818	-	-	-	+	+	-	+	-	+	+	+	-
Hygrotus inaequalis (Fabricius, 1776)	+	-	-	-	-	-	-	-	-	-	-	-
Hyphydrus sp. (larva)	+	+	-	+	+	-	+	-	+	-	+	+
Ilybius sp. (larva)	-	+	+	-	-	-	-	-	-	-	-	-
Laccophilus sp. (larva)	+	+	+	+	+	+	+	+	+	-	+	+
Liopterus atriceps (Sharp, 1882)	+	-	+	+	+	-	-	+	+	+	+	+
Porhydrus sp. (larva)	+	-	-	+	+	-	+	-	+	+	+	+
Rhantus sp. (larva)	+	-	+	-	-	+	-	-	-	-	+	-
F. Erirhinidae												
Stenopelmus rufinasus Gyllenhal, 1835	+	-	-	-	-	-	-	-	-	+	-	-
F. Gyrinidae												
Gyrinus caspius Ménétries, 1832	-	-	-	-	+	-	+	-	+	-	-	-
Gyrinus dejeani Brullé, 1832	-	-	-	-	+	-	+	-	+	-	-	-
F. Haliplidae												
Haliplus sp. (larva)	-	-	-	-	-	-	-	-	-	+	-	-
F. Helophoridae												
Helophorus cf. *lapponicus* Thomson, 1853	-	+	-	-	-	-	-	-	-	-	+	-
F. Hydraenidae												
Hydraena (Hydraena) sp.	-	-	-	+	+	-	-	-	-	-	-	-
F. Hydrophilidae												
Anacaena lutescens (Stephens, 1829)	-	-	-	-	-	+	-	-	-	-	-	-
Berosus sp. (larva)	+	+	-	+	+	-	+	-	+	+	+	+
Enochrus sp. (larva)	+	+	-	-	+	-	-	+	-	-	-	-
Helochares sp. (larva)	-	-	-	+	-	-	-	-	-	-	-	-
Hydrobius sp. (larva)	+	+	+	+	+	+	+	+	+	-	+	-
F. Hygrobiidae												
Hygrobia hermanni (Fabricius, 1775)	-	+	-	-	-	-	+	+	-	+	-	+
F. Noteridae												
Noterus laevis Sturm, 1834	-	-	-	+	-	-	+	-	-	-	+	-
F. Scirtidae												
Cyphon hilaris Nyholm, 1944	-	+	-	-	-	-	-	-	-	-	-	-

	B16	B23	B18	B9	B22	B14	B27	B26	B20	B12	B20b	B17
F. Tenebrionidae												
Alleculinae indet. (larva)	+	-	-	-	+	-	-	-	-	-	+	-
O. Diptera												
F. Ceratopogonidae												
Ceratopogonidae indet.	+	-	-	-	-	-	-	-	-	-	-	-
F. Chaoboridae												
Chaoborus flavicans (Meigen, 1830)	+	-	+	+	+	+	+	+	+	+	+	+
F. Chironomidae												
Chironominae indet.	+	+	+	+	+	+	+	+	+	+	+	+
Orthocladiinae indet.	+	+	+	+	+	+	+	+	+	+	+	+
Tanypodinae indet.	+	+	+	+	+	+	+	+	+	+	+	+
F. Culicidae												
Culex pipiens Linnaeus, 1758	+	-	+	+	-	+	+	+	-	+	+	-
Culex theileri Theobald, 1903	+	-	+	+	-	+	-	+	-	+	+	-
Culiseta subochrea/annulata	+	+	-	-	+	+	-	-	-	-	+	+
F. Dixidae												
Dixella attica (Pandazis, 1933)	-	-	-	-	+	-	+	-	-	-	-	-
F. Ephydridae												
Ephydridae indet.	-	+	-	-	-	-	-	-	-	-	-	-
F. Limoniidae												
Dicranomyia/Atypophthalmus/Neolimonia	-	+	-	-	+	-	-	-	-	-	-	-
F. Tabanidae												
Tabanus sp.	-	-	-	-	-	-	-	-	-	+	-	-
Ph. Mollusca												
Cl. Bivalvia												
O. Veneroida												
F. Sphaeriidae												
Musculium lacustre (O. F. Muller, 1774)	-	-	-	-	-	-	-	-	+	-	-	-
Cl. Gastropoda												
O. Basommatophora												
F. Ancylidae												
Ferrissia sp.	-	-	-	-	-	-	+	+	-	-	+	-
F. Physidae												
Physella acuta Draparnaud, 1805	-	-	-	-	-	-	+	-	-	-	-	-
F. Planorbidae												
Gyraulus laevis (Alder, 1838)	+	+	-	+	+	-	+	+	+	-	+	+
Ph. Chordata												
Cl. Amphibia												
O. Anura												
F. Hylidae												
Hyla meridionalis/arborea	+	-	+	+	+	+	-	-	+	+	+	+
F. Pelobatidae												
Pelobates cultripes (Cuvier, 1829)	+	+	+	+	+	+	+	+	-	+	+	+
F. Ranidae												
Pelophylax perezi (López Seoane, 1885)	-	-	-	-	-	-	-	+	-	-	-	-
O. Urodela												
F. Salamandridae												
Lissotriton boscai (Lataste, 1879)	+	+	+	+	+	-	+	+	+	+	+	+
Pleurodeles waltl Michahelles, 1830	-	-	-	-	-	+	-	-	+	-	-	-
Triturus pygmaeus (Wolterstorff, 1905)	+	+	+	+	+	+	+	+	+	+	+	+
Taxa richness	**35**	**27**	**23**	**37**	**32**	**27**	**38**	**26**	**30**	**31**	**32**	**29**

APPENDIX 2

(Supporting information of Chapter 2)

SI Table 4.2.1. Macrofaunal active dispersers from the SEPN

	VM01	VM02	VM03	VM04	VM05	VM06	VM07	VM08	VM10	VM11	VM12
Aeshna mixta	0	0	0	1	0	0	0	1	0	0	1
Agabus **sp.**	1	1	0	0	1	0	1	0	0	0	0
Alleculinae **indet.**	1	0	0	0	0	0	0	0	0	0	1
Anacaena lutescens	0	1	0	0	0	0	0	0	0	0	0
Anisops sardeus	1	1	1	0	1	1	1	0	0	0	1
Berosus **sp.**	1	0	1	1	1	1	0	1	0	1	1
Bidessus goudoti	0	0	1	0	1	1	0	0	1	1	0
Ceratopogonidae **indet.**	1	0	0	0	0	0	0	0	0	0	0
Chaoborus flavicans	1	1	1	1	1	1	1	1	1	0	1
Chironominae **indet.**	1	1	1	1	1	1	1	1	1	1	1
Cloeon **gr.** ***dipterum***	1	1	1	1	1	1	0	1	1	0	1
Cloeon schoenemundi	0	0	0	0	0	0	0	0	0	1	0
Coenagrionidae **indet.**	1	0	0	0	0	0	0	0	0	0	0
Corixa affinis	0	1	0	1	0	1	0	0	1	0	0
Culex pipiens	1	1	1	1	0	1	1	0	1	0	0
Culex theileri	1	1	1	1	0	1	1	0	1	0	0
Culiseta subochrea	1	1	0	0	1	0	0	0	0	1	1
Cybister tripunctatus africanus	0	0	0	1	0	0	0	0	0	0	0
Cyphon hilaris	0	0	0	0	0	0	0	0	0	1	0
Dicranomyia **sp.**	0	0	0	0	0	0	0	0	0	1	1
Dixella attica	0	0	0	1	0	0	0	0	0	0	1
Dryops doderoi	0	0	0	1	0	0	0	0	0	0	0
Enochrus **sp.**	1	0	0	0	0	0	0	0	1	1	1
Ephydridae **indet.**	0	0	0	0	0	0	0	0	0	1	0
Gerris thoracicus	1	1	1	1	1	1	0	0	1	1	1
Graptodytes flavipes	1	1	1	0	0	1	1	0	0	0	0
Gyrinus caspius	0	0	0	0	0	0	0	1	0	0	0

SI Table 4.2.1 (cont.)

Gyrinus dejeani	0	0	0	1	0	0	0	1	0	0	1
Haliplus sp.	0	0	1	0	0	0	0	0	0	0	0
Helochares lividus	0	0	0	0	0	1	0	0	0	0	0
Helophorus lapponicus	0	0	0	0	0	0	0	0	0	1	0
Hesperocorixa linnaei	0	0	0	0	0	0	0	1	0	0	0
Hydraena sp.	0	0	0	0	0	1	0	0	0	0	1
Hydrobius sp.	1	1	0	1	1	1	1	1	1	1	1
Hydroporus tessellatus	1	0	1	0	0	1	1	1	1	0	0
Hydrovatus cuspidatus	0	0	1	1	0	1	0	1	0	0	1
Hygrobia hermanni	0	0	1	1	1	0	0	0	1	1	0
Hygrotus inaequalis	1	0	0	0	0	0	0	0	0	0	0
Hyla sp.	1	1	1	0	1	1	1	1	0	0	1
Hyphydrus aubei	1	0	0	1	1	1	0	1	0	1	1
Ilybius sp.	0	0	0	0	0	0	1	0	0	1	0
Laccophilus sp.	1	1	0	1	1	1	1	1	1	1	1
Lestes sp.	0	0	0	0	0	1	0	0	0	0	0
Liopterus atriceps	1	0	1	0	1	1	1	1	1	0	1
Lissotriton boscai	1	0	1	1	1	1	1	1	1	1	1
Noterus laevis	0	0	0	1	0	1	0	0	0	0	0
Notonecta glauca	0	1	0	0	0	0	0	0	0	1	0
Notonecta meridionalis	1	1	0	1	0	1	1	1	1	1	1
Orthocladiinae indet.	1	1	1	1	1	1	1	1	1	1	1
Pelobates cultripes	1	1	1	1	1	1	1	0	1	1	1
Pelophylax perezi	0	0	0	0	0	0	0	0	1	0	0
Plea minutissima	0	1	0	1	0	1	0	0	0	0	0
Pleurodeles waltl	0	1	0	0	0	0	0	1	0	0	0
Porhydrus sp.	1	0	1	1	1	1	0	1	0	0	1

SI Table 4.2.1 (cont.)

Ranatra linearis	0	0	0	0	1	0	0	0	0	0	0
***Rhantus* sp.**	1	1	0	0	0	0	1	0	0	0	0
Sigara lateralis	0	0	0	0	1	0	0	0	0	0	0
Sigara nigrolineata	0	0	0	0	0	0	0	0	0	1	0
Sigara scotti	0	0	1	0	0	0	0	0	0	0	0
Stenopelmus rufinasus	1	0	1	0	0	0	0	0	0	0	0
Sympetrum fonscolombii	0	0	1	1	1	1	1	1	1	1	1
Sympetrum striolatum	1	0	1	1	1	1	0	0	0	0	0
***Tabanus* sp.**	0	0	1	0	0	0	0	0	0	0	0
***Tanypodinae* indet.**	1	1	1	1	1	1	1	1	1	1	1
Trichocorixa verticalis	0	1	0	0	0	0	0	0	0	0	0
Triturus pygmaeus	1	1	1	1	1	1	1	1	1	1	1

SI Table 4.2.2. Macrofaunal passive dispersers from the SEPN

	VM01	**VM02**	**VM03**	**VM04**	**VM05**	**VM06**	**VM07**	**VM08**	**VM10**	**VM11**	**VM12**
Dina lineata	0	0	0	1	0	0	0	0	0	0	0
Dugesiidae indet.	0	0	1	1	1	1	0	1	0	0	0
Eiseniella tetraedra	0	0	0	0	0	0	0	0	0	0	1
Enchytraeidae indet.	1	1	1	1	1	1	0	1	1	1	0
***Ferrissia* sp.**	0	0	0	1	0	0	0	0	1	0	0
***Gyraulus* sp.**	1	0	0	1	1	1	0	1	1	1	1
***Hydra* sp.**	0	0	0	0	0	0	0	1	0	0	0
Musculium lacustre	0	0	0	0	0	0	0	1	0	0	0
Physa acuta	0	0	0	1	0	0	0	0	0	0	0
***Piona* sp.**	0	0	0	1	1	1	0	1	0	0	1
Rhabdocoela indet.1	0	0	1	1	1	0	1	1	1	0	0
***Tiphys* sp.**	1	1	1	1	1	1	0	0	0	0	0
Tubificidae indet. (with setae)	0	0	0	0	0	0	1	0	0	0	0

SI Table 4.2.3. Plants from the SEPN

	VM01	VM02	VM03	VM04	VM05	VM06	VM07	VM08	VM10	VM11	VM12
Anagallis arvensis	1	1	0	1	1	0	1	1	0	1	0
Agrostis stolonifera	1	1	1	1	1	1	1	1	1	1	1
Alisma lanceolatum	0	0	0	0	0	0	1	0	0	0	0
Anagallis tenella	1	1	0	1	1	0	0	1	1	1	1
Apium nodiflorum	0	0	0	0	0	0	1	0	0	0	0
Avena sterilis	0	0	0	0	0	0	1	0	0	0	0
Baldellia ranunculoides	1	1	1	1	1	1	1	1	1	1	1
Bolboschoenus maritimus	0	1	1	0	0	1	0	0	0	0	0
Briza maxima	1	0	0	0	1	0	0	1	0	0	1
Briza minor	1	0	0	0	1	0	0	0	0	0	0
Callitriche brutia	0	1	1	0	1	0	0	0	0	0	0
Callitriche stagnalis	0	0	0	0	1	1	1	0	0	0	0
Carex sp.	0	0	0	0	1	0	0	0	0	0	0
Caropsis verticillato-inundata	0	0	0	0	0	0	0	0	0	0	1
Carpobrotus edulis	0	1	0	0	0	0	0	0	0	0	0
Carum verticillatum	0	1	1	0	0	0	0	1	1	1	1
Centaurium maritimum	0	0	0	0	0	0	0	1	0	0	0
Chamaemelum mixtum	0	0	0	1	0	0	0	0	0	0	1
Chamaemelum nobile	0	0	0	0	0	0	0	0	1	0	0
Cynodon dactylon	0	1	1	0	1	0	0	1	1	1	1
Cistus psilosepalus	0	0	0	0	0	0	0	1	0	0	0
Cyperus longus	0	0	0	0	0	0	0	0	0	0	0
Dittrichia viscosa	0	1	0	0	1	0	1	1	1	1	1
Eleocharis multicaulis	1	0	1	0	0	0	0	1	1	0	1
Eleocharis palustris	1	1	0	0	1	0	0	1	0	0	0
Eryngium corniculatum	1	1	0	0	0	0	0	0	0	0	0
Euphorbia exigua	0	0	0	0	0	0	0	0	0	0	1

SI Table 4.2.3 (cont.)

Festuca arundinacea	0	0	0	0	0	0	0	0	1	0	0
Galium debile	0	0	0	0	1	0	1	1	1	1	1
Galium palustre	1	1	1	1	1	0	0	0	0	0	0
Glyceria declinata	0	0	0	0	1	0	0	0	0	0	0
Holcus lanatus	0	0	0	0	0	0	1	1	0	0	0
Hydrocotyle vulgaris	1	1	1	1	1	0	0	1	1	1	1
Hypericum elodes	1	1	0	0	1	0	0	1	1	1	1
Hypericum humifusum	0	0	0	0	0	0	0	1	0	0	1
***Iris* sp.**	0	0	0	0	1	0	0	0	0	0	0
Isoetes velatum	0	0	0	0	1	0	0	0	0	0	0
Isolepis cernua	1	1	0	0	1	0	0	1	1	0	0
Isolepis pseudosetacea	0	1	0	0	1	0	0	0	0	0	0
Isolepis fluitans	0	0	0	1	0	0	0	1	1	0	1
Juncus articulatus	0	0	0	0	0	1	1	0	0	0	0
Juncus bulbosus	0	1	0	0	0	0	0	1	0	0	1
Juncus capitatus	0	0	0	0	0	0	0	0	1	0	0
Juncus effusus	0	0	1	0	1	1	0	0	0	0	1
Juncus emmanuelis	0	1	1	0	1	0	0	1	1	0	0
Juncus heterophyllus	1	1	1	1	1	0	1	1	1	0	1
Juncus pygmaeus	0	1	0	0	0	0	0	0	0	0	0
Leontodon taraxacoides	1	1	1	1	0	0	1	1	1	1	1
Lobelia urens	0	0	0	0	0	0	0	1	0	0	1
Lotus hispidus	0	1	0	1	1	0	0	0	0	0	0
Lythrum hyssopifolia	0	0	1	0	0	0	0	0	0	0	0
Lythrum junceum	0	1	1	1	1	1	0	1	0	0	1
Mentha pulegium	1	0	0	1	0	0	0	1	0	0	0
Mentha suaveolens	0	0	0	0	0	0	1	0	0	0	0

SI Table 4.2.3 (cont.)

Myosotis debilis	0	0	0	0	0	0	0	1	0	0	0
Myosotis laxa	0	0	0	0	0	0	0	1	0	0	0
Myriophyllum alterniflorum	0	1	0	0	0	0	0	0	0	0	0
Ornithopus pinnatus	0	0	1	0	0	0	0	0	0	0	1
Panicum repens	0	1	1	0	0	0	0	1	1	1	1
Parentucellia viscosa	0	0	0	0	0	0	0	1	1	1	0
Paspalum paspalodes	0	0	0	0	0	0	0	1	0	1	0
Pinguicula lusitanica	0	1	0	0	0	0	0	1	0	0	0
Plantago coronopus	0	0	1	1	0	0	0	0	0	0	1
Pulicaria paludosa	0	0	0	0	0	0	1	0	0	0	0
Ranunculus flammula	0	0	0	0	0	0	0	1	0	0	0
Ranunculus longipes	0	0	0	0	1	0	0	0	0	0	0
Ranunculus paludosus	0	0	0	1	0	0	1	0	0	0	0
Ranunculus peltatus	1	1	1	0	1	1	1	0	0	0	0
Samolus valerandi	0	0	0	0	1	0	0	0	0	0	0
Schoenoplectus lacustris	0	1	1	0	1	0	0	1	0	0	0
Scirpoides holoschoenus	0	0	0	1	0	0	0	1	0	1	1
Scorpiurus vermiculatus	0	0	0	0	1	0	0	0	0	0	0
Serapias cordigera	0	1	0	0	0	0	0	0	0	0	0
Silene laeta	0	0	0	1	1	0	1	1	0	0	0

SI Table 4.2.4. Macrofaunal active dispersers from the LEPN

	GG01	GG02	GG03	GG04	GG05	GG06	GG07	GG08	GG09	GG10	GG11
Acilius **sp.**	0	1	0	0	0	0	0	1	1	0	0
Agabus nebulosus	0	0	1	1	1	1	0	1	1	0	1
Anopheles maculipennis	1	1	0	0	1	0	0	0	1	0	0
Bagous **sp.**	1	0	1	0	1	1	0	0	1	1	0
Berosus affinis	1	1	0	1	1	0	1	1	1	0	1
Berosus signaticollis	0	0	1	0	1	0	1	0	0	0	0
Bidessus goudoti	1	1	1	1	1	0	1	1	1	1	1
Bufotes balearicus	0	0	1	1	1	1	1	1	1	1	1
Chironominae indet.	1	1	1	1	1	1	1	1	1	1	0
Cloeon **gr.** ***dipterum***	0	1	0	1	0	0	1	0	0	0	0
Colymbetes **sp.**	1	0	0	0	1	0	0	0	0	0	1
Corixa **sp.**	1	1	1	1	1	1	1	1	1	1	1
Culex hortensis	0	0	0	0	1	0	0	0	0	0	0
Culicoides **sp.**	1	0	0	0	1	0	0	1	1	0	0
Culiseta fumipennis	0	0	0	0	1	0	1	0	0	0	0
Culiseta litorea	1	0	0	0	0	0	0	0	0	0	0
Dryops striatellus	1	1	0	0	1	0	1	1	1	1	1
Dytiscus **sp.**	0	0	0	1	0	1	0	1	0	1	0
Empididae indet.	0	0	0	0	0	1	0	0	0	0	0
Enochrus quadripunctatus	0	0	0	0	1	0	0	1	0	1	0
Gerris thoracicus	0	1	1	1	0	0	1	1	0	1	1
Graptodytes flavipes	1	1	0	1	0	1	1	0	1	0	1
Habrophlebia consiglioi	0	0	0	0	1	0	0	0	0	0	0
Haliplus guttatus	0	0	0	1	0	0	0	0	0	0	0
Haliplus lineatocollis	1	1	1	0	1	0	1	0	1	0	1
Helochares lividus	1	0	0	0	0	0	0	0	0	0	0
Helophorus alternans	1	1	0	0	1	0	0	0	0	0	0

SI Table 4.2.4 (cont.)

Helophorus asturiensis	0	0	0	0	1	0	0	0	0	0	0
Helophorus* gr. *maritimus	0	0	0	0	1	0	0	1	0	0	0
Helophorus subarcuatus	0	0	0	1	1	0	0	1	1	1	0
***Hydrellia* sp.**	0	0	0	0	1	0	0	0	0	0	1
***Hydrobius* sp.**	0	0	0	0	1	0	0	0	0	0	0
Hydrochus flavipennis	0	1	0	0	1	1	1	0	1	1	0
Hydroglyphus geminus	1	0	0	0	0	0	0	0	1	0	0
Hydroporus memnonius	0	0	0	0	1	0	0	0	0	0	0
Hydroporus pubescens	1	1	1	1	1	0	0	1	1	0	1
Hydroporus tessellatus	1	1	0	0	0	0	0	0	0	0	0
Hygrobia hermanni	0	0	0	1	0	1	0	0	0	0	0
***Hygrotus* sp.**	0	0	0	0	0	0	0	0	0	1	0
Hyla sarda	0	1	0	1	1	1	1	1	0	1	1
Sciomyzidae indet.	0	0	0	0	0	1	0	0	1	1	0
Laccophilus minutus	1	1	0	1	1	1	1	1	1	1	1
Lestes barbarus	1	1	1	1	1	1	1	1	1	1	1
Limnephilus vittatus	1	1	0	1	1	1	1	1	1	1	1
***Liopterus* sp.**	0	0	0	1	1	0	0	1	1	0	1
Notonecta meridionalis	1	1	1	1	1	1	1	1	1	1	1
Ochthebius dilatatus	1	1	0	0	1	1	0	1	1	1	1
Orthocladiinae indet.	1	1	1	1	1	1	1	1	1	1	1
***Phytobius* sp.**	0	0	0	0	0	1	0	0	0	1	1
Plea minutissima	1	0	0	0	0	0	1	1	1	0	0
Sigara lateralis	0	1	1	1	1	0	0	1	1	1	1
Sigara nigrolineata	0	0	0	1	0	0	1	1	1	1	1
Sympetrum striolatum	0	1	0	1	0	0	1	1	1	1	1
***Tabanus* sp.**	0	0	0	1	1	0	0	1	1	1	0
Tanypodinae indet.	1	0	1	1	1	1	1	1	1	1	1

SI Table 4.2.5. Macrofaunal passive dispersers from the LEPN

	GG01	GG02	GG03	GG04	GG05	GG06	GG07	GG08	GG09	GG10	GG11
Ancylus sp.	0	0	0	0	0	0	0	0	1	0	0
Arrenurus papillator	0	1	0	0	1	0	0	0	0	0	0
Bothromesostoma personatum	0	0	0	1	0	0	1	0	1	1	0
Chirocephalus salinus	0	0	1	0	0	1	0	0	0	1	0
Eylais extendens	1	1	0	1	1	0	1	1	1	1	1
Dugesiidae indet.	0	0	0	0	0	0	0	0	0	1	0
Enchytraeidae indet.	1	0	1	0	1	1	1	0	0	0	1
***Achaeta* sp.**	0	0	0	0	0	1	0	0	0	0	0
Lumbriculidae indet.	1	1	0	1	0	0	0	0	0	0	1
Lumbricidae indet.	0	0	0	0	0	0	0	0	1	0	0
Naididae indet.	0	0	0	0	0	1	0	0	1	0	0
Tubificidae indet. (with setae)	1	1	1	1	1	1	1	1	1	0	1
Tubificidae indet. (without setae)	0	0	0	1	0	0	1	0	0	0	1
Galba truncatula	1	0	1	0	0	0	1	0	0	0	0
Gieysztoria beltrani	1	1	0	1	1	1	1	1	1	1	1
***Gyraulus* sp.**	0	1	0	1	1	1	1	1	1	1	1
Gyratrix hermaphroditus	0	0	0	0	0	0	0	1	1	0	0
Hydrachna goldfeldi	0	0	0	0	0	0	0	1	0	0	0
***Hydrachna* sp.1**	0	0	1	0	0	0	0	0	0	1	0

SI Table 4.2.5 (cont.)

Lepidurus apus	0	0	0	0	0	1	0	0	0	0	0
Rhabdocoela indet.1	0	1	0	1	1	1	1	1	1	1	0
Rhabdocoela indet.2	1	0	0	1	0	1	0	1	1	0	1
Rhabdocoela indet.3	0	0	1	0	0	0	0	0	0	0	1
Rhabdocoela indet.5	0	0	0	1	0	0	1	1	0	1	0
Rhabdocoela indet.7	0	0	0	0	1	1	1	1	0	0	0
Rhabdocoela indet.9	0	0	0	0	1	0	1	0	0	0	0
***Phaenocora* sp.**	1	1	1	1	1	1	1	1	1	1	0

SI Table 4.2.6. Plants from the LEPN

	GG01	**GG02**	**GG03**	**GG04**	**GG05**	**GG06**	**GG07**	**GG08**	**GG09**	**GG10**	**GG11**
Alisma plantago-aquatica	0	0	0	1	0	0	0	0	0	0	0
Alopecurus bulbosus	1	1	0	1	1	1	1	1	1	1	1
Anthoxanthum aristatum	0	0	0	0	0	1	1	0	0	0	0
Antinoria insularis	1	1	1	1	0	0	1	1	0	1	1
Apium crassipes	1	1	1	1	1	1	1	1	1	1	1
Asphodelus ramosus	0	0	0	0	1	1	1	0	1	1	1
Baldellia ranunculoides	1	1	1	1	0	1	1	1	1	1	1
Bellis annua	1	1	1	0	0	1	1	1	1	1	1
Briza maxima	0	0	0	0	0	1	0	0	0	1	0
Briza minor	0	0	0	0	1	1	0	0	1	1	0
Bromus hordeaceus	1	0	0	1	0	0	1	0	1	1	0
Bulliarda vaillantii	0	1	0	0	0	0	0	0	0	0	1

SI Table 4.2.6 (cont.)

Callitriche brutia	0	0	1	0	0	0	0	1	0	0	1
Callitriche stagnalis	1	1	0	0	0	0	1	1	0	0	0
Carex divisa	0	0	0	1	1	0	1	1	0	1	0
Centaurium erythraea	0	0	0	1	0	0	1	1	1	1	0
Centaurium maritimum	1	0	0	0	0	1	0	0	0	1	0
Chamaemelum fuscatum	1	0	1	1	1	1	1	1	1	1	1
***Chara* sp.**	1	0	1	0	0	0	0	0	0	0	0
Convolvulus arvensis	0	0	0	1	0	0	1	0	0	0	0
Cynara cardunculus	0	0	0	1	0	0	0	0	0	0	0
Cynodon dactylon	1	1	0	0	0	1	1	1	0	1	1
Cynosurus cristatus	0	0	0	1	1	1	0	0	0	0	0
Damasonium alisma	0	0	0	0	1	0	0	1	1	1	1
Dipsacus ferox	0	0	0	1	0	0	0	0	0	0	0
Dittrichia graveolens	0	0	0	1	0	0	1	0	0	1	0
Dittrichia viscosa	1	1	0	0	0	0	0	0	0	0	0
Echium plantagineum	0	0	0	1	0	1	1	0	1	0	0
Elatine macropoda	1	1	1	0	0	1	1	1	0	1	1
Eleocharis acicularis	1	0	1	0	0	1	1	0	0	0	1
Eleocharis palustris	0	0	1	1	1	0	1	1	0	1	1
Eryngium barrelieri	1	1	1	1	1	1	1	1	1	1	1
Eryngium corniculatum	1	1	1	0	0	1	1	1	1	1	1
Eudianthe laeta	1	0	0	1	0	1	1	0	1	1	1
Exaculum pusillum	1	1	1	1	0	1	1	1	1	1	1
Gastridium ventricosum	0	0	0	1	0	1	0	0	0	0	0
Gaudinia fragilis	0	0	0	1	0	1	0	0	1	1	1
Geranium molle	0	0	0	1	0	0	0	0	0	0	0
Glyceria spicata	1	1	1	1	1	0	1	1	0	1	0

SI Table 4.2.6 (cont.)

Hordeum hystrix	1	0	0	1	1	0	1	1	1	1	1
Hypochaeris achyrophorus	0	0	0	0	0	0	0	0	1	1	0
Hypochaeris radicata	0	0	0	0	0	0	1	0	0	0	0
Illecebrum verticillatum	1	0	0	0	0	1	1	1	1	1	0
Isoëtes gymnocarpa	0	1	0	0	1	1	1	1	1	1	1
Isoetes histrix	0	1	0	0	1	1	1	1	1	1	1
Isoëtes velata	1	1	1	1	1	1	1	1	1	1	1
Isolepis cernua	1	0	0	0	1	1	1	0	0	1	0
Juncus bufonius	1	1	0	1	1	1	1	1	1	0	1
Juncus heterophyllus	0	0	0	0	1	0	0	0	1	0	0
Juncus pygmaeus	1	1	1	1	1	1	1	1	1	1	1
Laurentia gasparrinii	1	0	1	0	0	0	1	0	1	1	1
Linum bienne	1	0	0	1	0	1	1	1	1	1	0
Linum trigynum	0	0	0	0	0	0	0	0	0	1	0
Littorella uniflora	0	0	1	0	0	0	0	0	0	0	0
Lolium multiflorum	0	0	0	1	0	0	1	0	0	0	0
Lotus angustissimus	1	1	0	0	1	1	1	1	1	1	1
Lotus edulis	1	0	0	0	0	0	0	0	0	0	0
Lotus hispidus	0	0	0	1	0	1	0	1	1	1	1
Lysimachia arvensis	0	0	0	1	0	0	0	0	0	1	0
Lysimachia foemina	0	0	0	0	1	1	1	1	1	1	1
Lythrum hyssopifolia	1	1	0	1	1	1	1	1	1	1	1
Medicago minima	0	0	0	0	0	0	0	1	0	0	0
Mentha pulegium	1	1	1	1	1	1	1	1	1	1	1
Middendorfia borysthenica	1	1	1	1	0	1	1	1	1	1	1
Myosotis sicula	1	1	1	1	0	1	1	1	1	1	1
Myriophyllum verticillatum	0	1	1	0	1	1	1	1	0	1	1

SI Table 4.2.6 (cont.)

Neoschischkinia pourretii	1	0	0	1	0	0	1	1	0	0	1
Oenanthe fistulosa	0	0	0	0	1	0	0	1	1	1	0
Oenanthe lisae	1	1	1	0	0	0	0	0	0	1	0
Oenanthe pimpinelloides	0	0	0	0	0	0	1	1	1	1	1
Oglifa gallica	0	0	0	0	0	1	0	0	0	0	0
Parentucellia viscosa	0	0	0	0	0	0	1	0	1	1	0
Pilularia minuta	1	1	1	0	0	1	1	1	1	1	1
Plantago coronopus	0	0	0	1	0	1	1	0	0	1	1
Plantago lanceolata	1	1	1	1	1	1	1	1	1	1	1
Poa infirma	1	0	0	0	0	0	1	0	0	0	0
Polypogon subspathaceus	1	1	1	1	1	1	1	1	1	1	1
Potentilla reptans	0	0	0	1	0	0	0	1	1	0	0
Pulicaria sicula	0	0	0	1	0	1	1	1	1	0	1
Ranunculus aquatilis	1	1	1	1	1	1	1	1	0	1	1
Ranunculus cordiger	0	0	0	1	1	1	0	1	1	1	0
Ranunculus flabellatus	0	0	0	0	0	0	1	0	0	0	0
Ranunculus macrophyllus	0	0	0	0	1	1	1	0	1	1	1
Ranunculus ophioglossifolius	0	1	0	1	1	1	1	1	1	1	1
Ranunculus revelieri	0	1	0	0	0	1	0	1	1	0	1
Ranunculus sardous	1	0	0	1	1	1	1	1	0	1	0
Ranunculus trichophyllus	1	0	1	0	0	0	0	0	0	0	0
Romulea ligustica	0	0	1	0	0	0	0	0	0	0	0
***Romulea requienii* Parl.**	1	0	0	0	1	0	0	0	0	1	0
Rumex pulcher	0	0	0	1	0	0	1	1	1	1	1
Scolymus hispanicus	0	0	0	1	0	0	0	0	0	0	0
Serapias lingua	0	0	0	0	0	1	0	0	1	0	0
Trifolium arvense	0	0	0	0	0	0	0	0	0	1	0

SI Table 4.2.6 (cont.)

Trifolium campestre	0	0	0	1	0	0	0	0	0	1	1
Trifolium michelianum	1	0	0	0	1	1	1	1	1	1	1
Trifolium micranthum	0	0	0	0	0	0	0	0	1	0	1
Trifolium nigrescens	0	0	0	0	1	0	1	0	1	0	1
Trifolium resupinatum	0	0	0	1	1	0	1	1	1	1	0
Trifolium subterraneum	1	0	0	0	1	1	1	0	1	1	0
Tuberaria lignosa	0	0	0	0	0	0	0	0	0	1	0
Vulpia ligustica	0	0	0	1	1	1	1	0	1	1	1
Vulpia myuros	1	0	0	0	0	0	0	0	0	0	0

SI Table 4.2.7. Mean and range of the variation in the environmental and geographic parameters from each pond network. Abbreviations are SEPN (small extent pond network), LEPN (large extent pond network), DIN (dissolved inorganic nitrogen), TIC (total inorganic carbon), DIC (dissolved inorganic carbon), DOC (dissolved organic carbon), TOC (total organic carbon), and DNP (distance to the nearest pond).

Parameter	Mean (Range)	
	SEPN	LEPN
Water temperature (°C)	21.6 (17.4-23.9)	18.2 (11.8-25.3)
Dyssolved oxygen (mg/L)	4.6 (2.8-6.6)	10.6 (8.1-13.4)
Conductivity (µS/cm)	778.9 (428.0-1274.7)	420 (238-614)
pH	6.54 (5.99-7.28)	9.47 (7.80-10.55)
Maximum depth (cm)	59.6 (30.0-106.0)	26.6 (14.0-59.0)
Ammonium (mg NH^{4+}-N/L)	0.013 (0.001-0.052)	0.032 (0.015-0.049)
Nitrite (mg NO^{2-}-N/L)	0.004 (0.003-0.006)	<0.001
Nitrate (mg NO^{3-} -N/L)	0.003 (0.002-0.009)	0.007 (0.000-0.067)
Phosphate (mg PO^{3-}_4 -P/L)	0.017 (0.004-0.096)	0.001 (0.001-0.003)
DIN (mg N/L)	0.02 (0.006-0.066)	18.147 (14.525-20.851)
TIC (mg C/L)	15.276 (5.231-75.190)	8.672 (4.431-11.430)
DIC (mg C/L)	13.873 (2.058-73.640)	7.386 (3.796-11.130)
DOC (mg C/L)	54.675 (40.140-79.440)	11.787 (7.619-19.980)
TOC (mg C/L)	57.779 (40.140-80.120)	12.548 (8.000-20.730)

SI Table 4.2.7 (cont.)

Parameter	Mean (Range)	
	SEPN	LEPN
Total Nitrogen (mg NT-N/L)	2.764 (1.874-3.798)	1.137 (0.695-1.726)
Total Phosphorus (mg PT-P/L)	0.132 (0.034-0.640)	0.095 (0.039-0.305)
Chlorophyll *a* (µg/L)	11.224 (0.271-40.868)	4.878 (1.199-14.424)
% Fulvic acids	63.085 (50.889-77.487)	21.504 (8.858-33.339)
Macrophyte biomass (g DW/cm^2)	0.194 (0.120-0.313)	0.008 (0.004-0.015)
Pond surface (m^2)	18637.7 (245.0-78652.0)	20171.5 (565.0-79990.0)
DNP (m)	179.6 (130.5-214.7)	305.5 (82.1-474.6)

SI Table 4.2.8. Mantel correlations between the environmental distance taking into account only the variables selected by the BIO-ENV analyses for each biotic group and network and the geographic distance. Abbreviations are SEPN (small extent pond network), LEPN (large extent pond network), AD (macrofaunal active dispersers), PD (macrofaunal passive dispersers) and PL (plants).

Pond network	Biotic group	R^2	p-value
SEPN	AD	0.208	0.077
	PD	0.188	0.112
	PL	0.144	0.162
LEPN	AD	0.138	0.109
	PD	0.059	0.241
	PL	0.083	0.251

SI Table 4.2.9. Regression parameters of the two regression models (linear and power-law) explored for the SEPN and LEPN. Acronyms stand for SEPN (small extent pond network), LEPN (large extent pond network), AD (macrofaunal active dispersers), PD (macrofaunal passive dispersers) and PL (plants).

Distance	Pond network	Group	R^2 Linear	R^2 power-law
Geographic	SEPN	AD	0.075	0.034
		PD	0.031	0.026
		PL	0.013	0.028
	LEPN	AD	-0.017	-0.018
		PD	-0.019	-0.017
		PL	0.007	-0.019
Environmental	SEPN	AD	0.247	0.232
		PD	0.094	0.070
		PL	-0.011	-0.018
	LEPN	AD	0.453	0.470
		PD	0.331	0.296
		PL	0.105	0.101

APPENDIX 3

(Supporting information of Chapter 3)

SI Table 4.3.1. Subset of beta regression models with $\delta \leq 2$. The model with the highest pseudo-R^2 is marked in bold.

LCBD		**Pseudo-R^2**	**LCBD-ad**		**Pseudo-R^2**
	betweenness + closeness + ECELS + pond size + network	**0.620**		degree	0.078
	pond size + ECELS + network	0.562		degree + pond size	0.106
	closeness + pond size +ECELS + network	0.587		closeness + degree	0.110
	betweenness + closeness + ECELS + network	0.579		**closeness + degree + pond size**	**0.150**
	degree + pond size + ECELS + network	0.578		pond size	0.029
LCBD-re	closeness + degree + network	0.378	**SCBD-prop**	closeness + network + closeness: network	0.586
	ECELS + network	0.341		**closeness + ECELS + PC1 + network + closeness: network**	**0.643**
	closeness + degree + ECELS + network	0.414		closeness + ECELS + network + closeness: network	0.613
	network	0.287		PC1 + closeness + network + closeness: network	0.605
	betweenness + closeness + degree + network	0.402		degree + closeness + network + closeness: network	0.600
	closeness + network	0.318			
	betweenness + closeness + degree + ECELS + network	**0.441**			
	closeness + ECELS + network	0.354			

SI Table 4.3.2. Results of the beta regression models with the highest pseudo-R^2 (the value for each model is included next to the response variables, in brackets). Abbreviations are SE (standard error), z value (the regression coefficient divided by its standard error), p (significance p-value), LCBD (local contribution to beta diversity), ECELS (Shallow lentic ecosystem conservation status), LCBD-re (proportion of the LCBD explained by replacement), LCBD-ad (proportion of the LCBD explained by abundance difference), PC1 (the first principal component of the principal component analysis) and SCBD-prop (proportion between the number of species with a value of SCBD above the mean value of the set of sampled ponds in a network to the total species richness of a pond).

	estimate	**SE**	**z value**	**p**
LCBD [0.620]				
(intercept)	-2.398	0.025	-97.538	**<0.001**
NW 2	0.198	0.035	5.672	**<0.001**
NW 3	-0.001	0.035	-0.035	0.972
NW 4	0.093	0.035	2.671	**0.008**
betweenness	0.031	0.016	1.925	0.054
closeness	-0.042	0.017	-2.515	**0.012**
ECELS	-0.038	0.014	-2.828	**0.005**
pond size	0.028	0.013	2.095	**0.036**
LCBD-re [0.441]				
(intercept)	-2.397	0.033	-72.181	**<0.001**
NW 2	0.196	0.047	4.146	**<0.001**
NW 3	-0.002	0.047	-0.037	0.971
NW 4	0.094	0.047	2.007	**0.045**
betweenness	0.029	0.021	1.347	0.178
closeness	-0.077	0.029	-2.684	**0.007**
degree	0.054	0.026	2.056	**0.040**
ECELS	-0.030	0.018	-1.680	0.093
LCBD-ad [0.150]				
(intercept)	-2.348	0.092	-25.425	**<0.001**
closeness	0.197	0.135	1.459	0.145
degree	-0.340	0.138	-2.454	**0.014**
pond size	0.143	0.087	1.635	0.102
SCBD-prop [0.643]				
(intercept)	-0.284	0.057	-5.000	**<0.001**
NW 2	-0.090	0.085	-1.057	0.290
NW 3	-0.521	0.083	-6.264	**<0.001**
NW 4	-0.398	0.084	-4.714	**<0.001**
ECELS	0.072	0.035	2.058	**0.040**
PC1	-0.034	0.018	-1.944	0.052
closeness	-0.116	0.061	-1.892	0.059
closeness: NW 2	-0.126	0.093	-1.361	0.173
closeness: NW 3	0.175	0.089	1.977	**0.048**
closeness: NW 4	0.346	0.089	3.907	**<0.001**

SI Table 4.3.3. Subset of linear models with δ≤2. The model with the highest adjusted-R^2 is marked in bold.

		Adjusted R^2
S-rar	**closeness + PC1 + network + closeness: network + PC1: network**	**0.694**
	closeness + pond size + network + closeness: network + pond size: network	0.689
	closeness + network + closeness: network	0.616
H	**closeness + network**	**0.282**
	closeness	0.192
TD	pond size + network + pond size: network	0.560
	betweenness + pond size + network + pond size: network	**0.561**
	degree + pond size + network + pond size: network	0.556
	ECELS + pond size + network + pond size: network	0.554
ATD	pond size + network + pond size: network	0.646
	closeness + pond size + network + pond size: network	**0.661**
	PC1 + pond size + network + pond size: network	0.648

SI Table 4.3.4. Results of the linear models with the highest adjusted-R^2 from the subset (the value for each model is included next to the response variables, in brackets). Abbreviations are SE (standard error), t value (the regression coefficient divided by its standard error), p (significance p-value), S-rar (rarefied species richness), PC1 (the first principal component of the principal component analysis), TD (taxonomic distinctness) and ATD (average taxonomic distinctness).

	estimate	**SE**	**t value**	**p**
S-rar [0.694]				
(intercept)	26.109	1.056	24.733	**<0.001**
NW 2	-6.238	1.566	-3.984	**<0.001**
NW 3	-4.432	1.493	-2.969	**0.006**
NW 4	4.391	1.526	2.877	**0.007**
closeness	6.943	1.113	6.241	**<0.001**
closeness: NW 2	-5.063	1.839	-2.753	**0.010**
closeness: NW 3	-7.028	1.641	-4.282	**<0.001**
closeness: NW 4	-5.163	1.656	-3.118	**0.004**
PC1	-1.809	0.692	-2.615	**0.013**
PC1: NW 2	2.300	0.955	2.408	**0.022**
PC1: NW 3	2.031	0.938	2.164	**0.038**
PC1: NW 4	3.121	0.878	3.556	**0.001**
H [0.282]				
(intercept)	2.071	0.107	19.330	**<0.001**
NW 2	1.907	0.159	-1.033	0.308
NW 3	1.829	0.152	-1.595	0.119
NW 4	2.246	0.155	1.131	0.265
closeness	0.208	0.058	3.585	**<0.001**
TD [0.561]				
(intercept)	67.198	2.330	28.838	**<0.001**
NW 2	4.088	3.456	1.183	0.245
NW 3	17.205	3.295	5.221	**<0.001**
NW 4	-2.973	3.369	-0.882	0.383
betweenness	-1.457	1.390	-1.048	0.302
pond size	2.388	2.609	0.915	0.366
pond size: NW 2	4.392	3.948	1.113	0.273
pond size: NW 3	-11.161	3.653	-3.055	**0.004**
pond size: NW 4	-3.654	3.637	-1.005	0.322

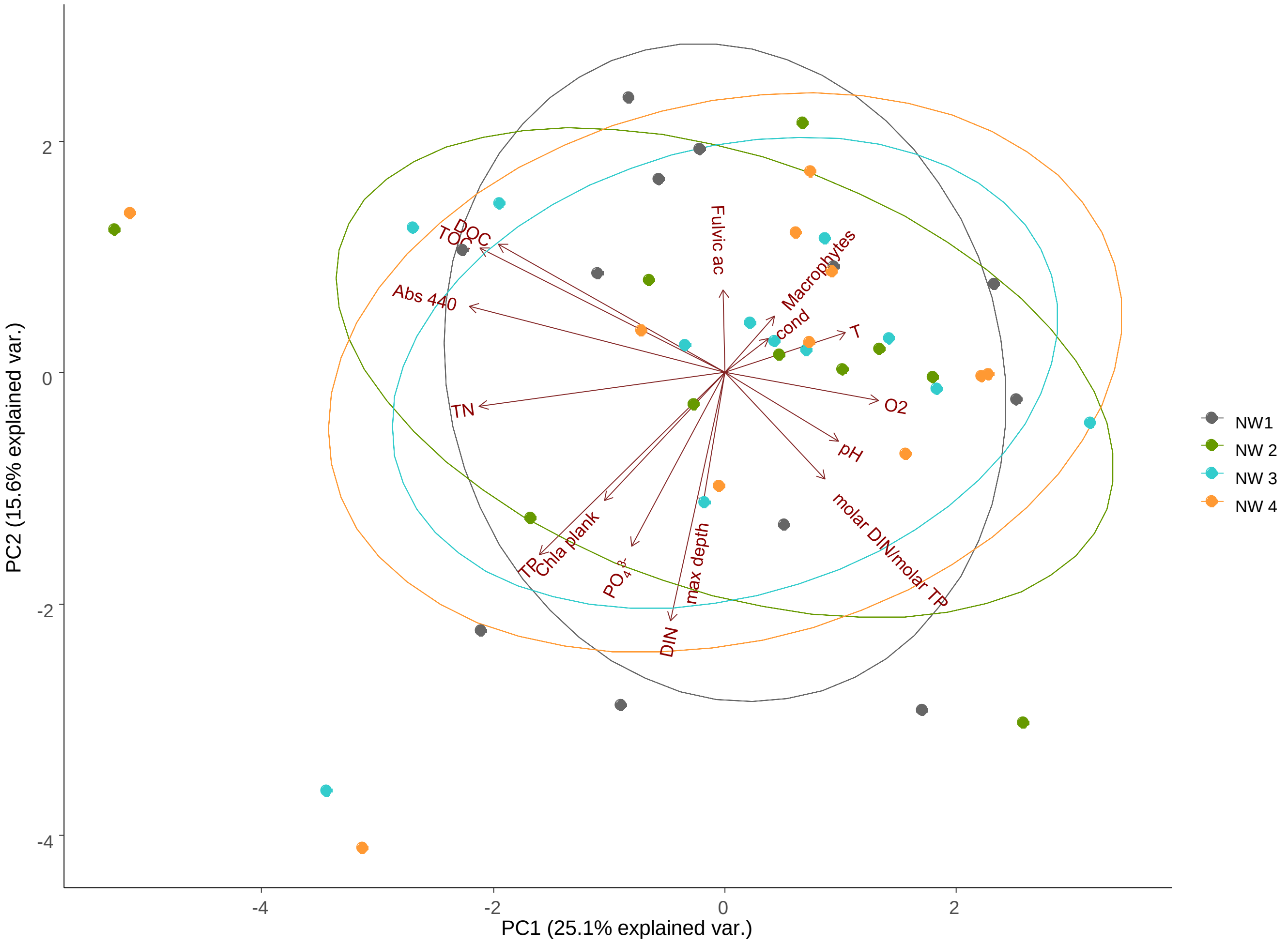
SI Table 4.3.4 (cont.)

	estimate	**SE**	**t value**	**p**
ATD [0.661]				
(intercept)	72.1284	0.7195	100.250	**<0.001**
NW 2	-4.1689	1.0672	-3.907	**<0.001**
NW 3	4.8826	1.0175	4.799	**<0.001**
NW 4	-0.1346	1.0404	-0.129	0.898
closeness	-0.6903	0.4281	-1.613	0.116
pond size	1.1471	0.7649	1.500	0.142
pond size: NW 2	0.7700	1.2152	0.634	0.530
pond size: NW 3	-3.2298	1.0724	-3.012	**0.005**
pond size: NW 4	-1.1869	1.1164	-1.063	0.295

SI Table 4.3.5. Mean, minimum and maximum values per pond network of the environmental parameters.

Parameter	NW 1			NW 2			NW 3			NW 4		
	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Maximum depth	94.67	28.00	200.00	34.10	15.00	91.00	62.33	30.00	106.00	26.64	14.00	59.00
Dissolved oxygen	8.54	4.40	12.17	6.43	3.61	9.21	4.58	2.82	6.61	10.61	8.08	13.35
Conductivity	251.86	112.10	727.00	28.25	15.08	55.00	746.53	390.00	1274.67	420.00	238.00	614.00
pH	7.50	6.81	8.57	6.52	5.62	7.84	6.51	5.99	7.28	9.47	7.80	10.55
Temperature	12.48	8.60	16.18	21.18	10.90	29.70	21.53	17.40	23.90	18.22	11.80	25.30
DIC	12.65	4.20	40.54	1.48	0.34	5.22	12.96	2.06	73.64	7.39	3.80	11.13
DOC	24.57	16.33	32.95	13.91	7.26	24.59	54.82	40.14	79.44	11.79	7.62	19.98
Phosphate	0.01	<0.01	0.01	<0.01	<0.01	<0.01	0.02	<0.01	0.10	<0.01	<0.01	<0.01
TIC	14.67	4.20	54.71	2.43	0.62	6.17	14.30	3.57	75.19	8.67	4.43	11.43
TOC	24.93	16.33	32.95	14.83	8.87	29.84	57.67	40.14	80.12	12.55	8.00	20.73
Absorbance at 440 nm	0.04	0.02	0.06	0.03	0.01	0.07	0.16	0.08	0.21	0.05	0.02	0.20
[TN-N]	1.72	1.28	2.88	0.99	0.46	1.84	2.77	1.87	3.80	1.14	0.69	1.73
[TP-P]	0.06	0.03	0.10	0.07	0.02	0.13	0.13	0.03	0.64	0.09	0.04	0.31
Fulvic acids	37.51	22.41	50.11	36.15	16.16	50.40	65.04	50.89	86.49	21.50	8.86	33.34
Macrophytes	0.06	0.01	0.13	0.01	0.00	0.04	0.19	0.12	0.31	0.01	0.00	0.01
Planktonic chlorophyll-*a*	9.92	0.99	33.48	4.10	0.84	13.46	10.47	0.27	40.87	4.88	1.20	14.42
DIN	0.05	0.02	0.15	0.06	0.03	0.13	0.02	0.01	0.07	0.04	0.02	0.11
Molar DIN/Molar TP	1.64	0.77	4.62	2.92	0.81	9.13	0.67	0.10	2.27	1.17	0.28	2.48

SI Figure 4.3.1. PCA plot showing the position of the sampled ponds in relation to the water characteristics. The ellipses separate the different networks.



SI Table 4.3.6. Checklist of the aquatic fauna found in the 4 pond networks surveyed. Abbreviations are NW (pond network), Ph. (Phylum), O. (Order), indet. (indeterminate), sp. (species), Cl. (Class), SCl. (Subclass), F. (Family), SF. (Subfamily), SO (Suborder), and SPh. (Subphylum). The presence of the taxon is marked with a 'X'. The symbol * denotes that the taxon includes both larvae and pupae and the symbol ◊ denotes the presence of individuals poorly developed.

Taxon	**NW 1**	**NW 2**	**NW 3**	**NW 4**
Ph. Cnidaria O. Anthomedusae				
Hydra sp.	X		X	
Ph. Platyhelminthes Cl. Turbellaria O. Rhabdocoela				
Rhabdocoela indet.1	X	X	X	X
Rhabdocoela indet.2	X			X
Rhabdocoela indet.3				X
Rhabdocoela indet.4	X			X
Rhabdocoela indet.5	X			X
Rhabdocoela indet.6				X
Rhabdocoela indet.7	X			
Rhabdocoela indet.8		X	X	
Rhabdocoela indet.9		X		
Gieysztoria sp.		X		
Microdalyellia sp.1		X		
Microdalyellia sp.2			X	
Phaenocora sp.	X	X		X
Bothromesostoma personatum			X	X
Dalyellia viridis	X			
Gieysztoria beltrani	X		X	X
Gieysztoria diadema	X		X	
Gyratrix hermaphroditus		X	X	X
O. Tricladida				
Dugesiidae indet.			X	X
Ph. Annelida Cl. Hirudinea F. Erpobdellidae				
Dina lineata			X	
F. Glossiphoniidae				
Helobdella stagnalis	X			
Scl. Oligochaeta F. Enchytraeidae				
Enchytraeidae indet.	X	X	X	X
Achaeta sp.		X		X
F. Lumbriculidae				
Lumbriculidae indet.	X	X	X	X
F. Lumbricidae				
Lumbricidae indet.				X
Eiseniella tetraedra			X	
F. Naididae				
Naididae indet.	X	X	X	X
F. Tubificidae				
Tubificidae indet.1 (with setae)	X	X	X	X
Tubificidae indet.2 (without setae)	X	X		X

Ph. Arthropoda Sph. Crustacea Cl. Malacostraca O. Isopoda				
Proasellus coxalis	X			
Cl. Branchiopoda O. Anostraca				
Chirocephalus diaphanus		X		
Chirocephalus salinus				X
O. Notostraca				
Lepidurus apus				X
Cl. Arachnida Scl. Acari Hydrachnidia F. Hydrachnidae				
Hydrachna sp.◊				X
Hydrachna goldfeldi				X
F. Pionidae				
Piona sp.◊	X	X	X	
Tiphys sp.◊			X	
Tiphys ornatus	X			
F. Arrenuridae				
Arrenurus papillator				X
F. Eylaidae				
Eylais extendens				X
F. Hydryphantidae				
Parathyas inepta		X		
O. Araneae F. Lycosidae				
Pirata piratula		X		
Cl. Insecta O. Odonata F. Aeshnidae				
Aeshna affinis	X			
Aeshna mixta			X	
Anax imperator	X			
F. Coenagrionidae				
Coenagrionidae indet.◊			X	
Coenagrion sp.◊	X			
Enallagma cyathigerum	X			
Erythromma lindeni	X			
Ischnura sp.◊	X			
F. Lestidae				
Lestes barbarus	X			X
Lestes dryas		X		
Lestes sp.◊			X	

F. Libellulidae				
Crocothemis erythraea	X			
Sympetrum flaveolum		X		
Sympetrum fonscolombii			X	
Sympetrum meridionale/striolatum	X		X	X
O. Ephemeroptera F. Baetidae				
Baetidae indet.◊		X		
Cloeon gr. *dipterum*	X		X	X
Cloeon schoenemundi			X	
O. Ephemeroptera F. Leptophlebidae				
Habrophlebia consiglioi				X
O. Hemiptera SO. Heteroptera F. Corixidae				
Corixa sp.◊				X
Corixa affinis			X	
Corixa panzeri	X			
Corixa punctata	X			
Hesperocorixa linnaei			X	
Hesperocorixa moesta	X			
Sigara dorsalis	X			
Sigara lateralis	X	X	X	X
Sigara limitata	X			
Sigara nigrolineata			X	X
Sigara scotti			X	
Trichocorixa verticalis			X	
F. Gerridae				
Gerris argentatus	X			
Gerris gibbifer	X	X		
Gerris thoracicus	X		X	X
F. Nepidae				
Ranatra linearis	X		X	
F. Notonectidae				
Anisops sardeus			X	
Notonecta glauca			X	
Notonecta meridionalis	X	X	X	X
F. Pleidae				
Plea minutissima	X		X	X
F. Saldidae				
Saldula sp.1 ◊	X			
Saldula sp.2 ◊	X			
O. Coleoptera F. Curculionidae				
Bagous sp. (larvae)				X
Phytobius sp. (larvae)				X
Stenopelmus rufinasus			X	

F. Dryopidae				
Dryops algiricus	X			
Dryops auriculatus		X		
Dryops doderoi			X	
Dryops striatellus	X			X
F. Dytiscidae				
Acilius sp. (larvae)				X
Agabus sp. (larvae)		X	X	
Agabus bipustulatus	X			
Agabus nebulosus	X			X
Bidessus goudoti	X		X	X
Colymbetes sp. (larvae)				X
Colymbetes fuscus	X			
Cybister lateralimarginalis	X			
Cybister tripunctatus africanus			X	
Dytiscus sp. (larvae)		X		X
Dytiscus circumflexus	X			
Graptodytes bilineatus	X			
Graptodytes flavipes	X		X	X
Hydroglyphus geminus	X			X
Hyphydrus aubei	X		X	
Hydroporus memnonius				X
Hydroporus necopinatus necopinatus		X		
Hydroporus pubescens	X			X
Hydroporus tessellatus	X		X	X
Hydroporus vagepictus	X			
Hydrovatus cuspidatus			X	
Hygrotus sp. (larvae)				X
Hygrotus impressopunctatus	X			
Hygrotus inaequalis			X	
Hygrotus marklini		X		
Ilybius sp. (larvae)	X		X	
Ilybius albarracinensis		X		
Laccophilus sp. (larvae)			X	
Laccophilus hyalinus	X			
Laccophilus minutus				X
Liopterus sp. (larvae)				X
Liopterus atriceps			X	
Liopterus haemorrhoidalis	X			
Porhydrus sp. (larvae)	X		X	
Rhantus sp. (larvae)	X		X	
F. Gyrinidae				
Gyrinus caspius			X	
Gyrinus dejeani	X		X	

F. Haliplidae				
Haliplus sp. (larvae)			X	
Haliplus guttatus	X			X
Haliplus lineatocollis	X			X
F. Helophoridae				
Helophorus alternans	X			X
Helophorus aquaticus		X		
Helophorus asturiensis	X			X
Helophorus brevipalpis	X	X		
Helophorus discrepans		X		
Helophorus flavipes		X		
Helophorus lapponicus			X	
Helophorus gr. maritimus				X
Helophorus subarcuatus				X
F. Hydraenidae				
Hydraena sp. (larvae)			X	
Ochthebius dilatatus				X
F. Hydrochidae				
Hydrochus angustatus	X			
Hydrochus flavipennis				X
Hydrochus smaragdineus	X			
F. Hydrophilidae				
Anacaena lutescens	X	X	X	
Berosus sp. (larvae)			X	
Berosus affinis	X			X
Berosus signaticollis	X	X		X
Berosus cf. *luridus*		X		
Enochrus sp. (larvae)			X	
Enochrus fuscipennis		X		
Enochrus nigritus	X			
Enochrus quadripunctatus	X			X
Hydrobius sp. (larvae)			X	X
Hydrobius fuscipes	X			
Helochares lividus	X		X	X
Hydrochara caraboides	X			
Laccobius sp. (larvae)	X			
Limnoxenus niger	X			
F. Hygrobiidae				
Hygrobia hermanni	X		X	X
F. Noteridae				
Noterus clavicornis	X			
Noterus laevis	X		X	
F. Scirtidae				
Cyphon sp. (larvae)	X			
Cyphon hilaris			X	

F. Tenebrionidae				
Alleculinae indet. (larvae)			X	
O. Diptera F. Ceratopogonidae				
Ceratopogonidae indet.*			X	
Alluaudomyia sp.*	X			
Bezzia sp.*	X			
Culicoides sp.*	X	X		X
Palpomyia sp.*	X			
F. Chaoboridae				
Chaoborus flavicans	X		X	
F. Chironomidae SF. Chironominae				
Dicrotendipes sp.*	X			
Endochironomus sp.*	X			
Glyptotendipes sp.*	X			
Kiefferulus sp.*	X			
Polypedilum sp.*	X	X		
Paratanytarsus sp.*		X		X
Tanytarsus sp.*	X			
Zavreliella sp.*	X			
Chironomus alpestris		X		
Chironomus piger			X	
Chironomus prasinus	X			
Chironomus riparius	X			
Micropsectra lindrothi	X	X		X
Microtendipes chloris	X			X
Microtendipes gr pedellus			X	
Parachironomus gr. arcuatus	X		X	X
Paratanytarsus grimmii	X		X	
F. Chironomidae SF. Orthocladiinae				
Corynoneura sp.*		X	X	X
Cricotopus sp.*		X	X	
Gymnometriocnemus sp.*		X		
Limnophyes sp.*	X	X		
Metriocnemus sp.*		X		
Corynoneura carriana	X			
Cricotopus sylvestris	X			X
Psectrocladius (P.) limbatellus	X	X		X
Psectrocladius (A.) obvius	X	X		
Psectrocladius (A.) platypus			X	X
Psectrocladius (P.) psilopterus		X		
Psectrocladius (P.) gr. sordidellus	X		X	X
F. Chironomidae SF. Podominae				
Lasiodiamesa sphagnicola		X		

F. Chironomidae SF. Tanypodinae				
Ablabesmyia sp.*	X	X		
Ablabesmyia monilis			X	
Macropelopia sp.*		X	X	
Macropelopia nebulosa	X			X
Procladius choreus	X	X	X	X
Psectrotanypus varius	X			
Xenopelopia falcigera	X		X	
Zavrelimyia barbatipes	X		X	
F. Culicidae				
Anopheles maculipennis				X
Culiseta fumipennis				X
Culiseta litorea	X			X
Culiseta subochrea			X	
Culex hortensis				X
Culex pipiens			X	
Culex theileri			X	
Ochlerotatus pullatus		X		
Ochlerotatus surcoufi		X		
F. Dixidae				
Dixella attica			X	
Dixella autumnalis	X			
F. Empididae				
Empididae indet.*				X
F. Ephydridae				
Ephydridae indet. (pupae)			X	
Hydrellia sp.*	X	X		X
F. Limoniidae				
Dicranomyia sp.*			X	
F. Scatophagidae				
Scathophagidae indet. 1*		X		
Scathophagidae indet. 2*		X		
F. Sciomyzidae				
Sciomyzidae indet.*				X
F. Tabanidae				
Tabanus sp.*			X	X
F. Tipulidae				
Prionocera sp.*		X		
O. Trichoptera F. Limnephilidae				
Limnephilus sp.1◊	X			
Limnephilus sp.2◊	X			
Limnephilus bipunctatus		X		
Limnephilus coenosus		X		
Limnephilus cf. *stigma*		X		
Limnephilus vittatus		X		X

O. Trichoptera F. Polycentropodidae				
Holocentropus stagnalis	X			
Ph. Mollusca Cl. Gastropoda F. Lymnaeidae				
Galba truncatula	X	X		X
Stagnicola palustris	X			
F. Physidae				
Physa acuta	X		X	
F. Planorbidae				
Ancylus sp.◊				X
Ferrissia sp.◊	X		X	
Gyraulus sp.◊	X		X	X
Gyraulus crista	X			
Hippeutis complanatus	X			
Cl. Bivalvia F. Sphaeriidae				
Musculium lacustre			X	
Pisidium casertanum		X		
Ph. Chordata Cl. Amphibia O. Anura				
Bufotes balearicus				X
Discoglossus pictus	X			
Epidalea calamita	X			
Hyla molleri/meridionalis			X	
Hyla sarda				X
Pelobates cultripes			X	
Pelodytes punctatus	X			
Pelophylax perezi			X	
Rana temporaria		X		
O. Caudata				
Lissotriton boscai			X	
Pleurodeles waltl			X	
Triturus marmoratus	X			
Triturus pygmaeus			X	

Icons that appear in the footnotes in order of appearance are: “frog” by sarah and “pond” by Serhii Smirnov (Introduction); “knowledge” by Payungkhead Im-anong (Hypotheses and Objectives); “fishing net” by Gan Khoon Lay (General Methodology); “giant water scavenger beetle” by Christine Reynolds (Chapter 1); “negative regression graph” by Aenne Brielmann (Chapter 2); “network” by Rflor (Chapter 3); “idea” by Adrien Coquet (General Discussion); “idea” by Priyanka (Conclusions); “books” by mikicon (References); “paper clip” by Vectors Point (Appendix 1, 2 and 3) from **The Noun Project (thenounproject.com/)**.

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