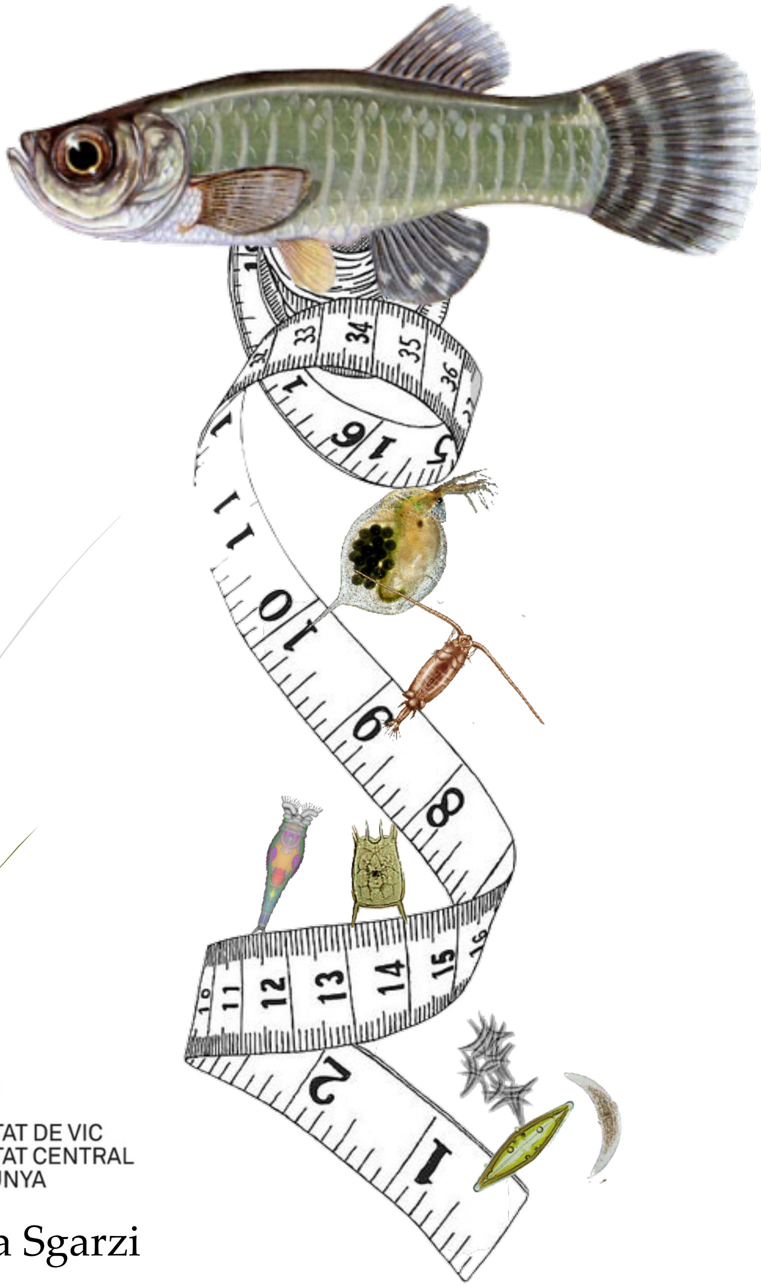
PhD THESIS

Environmental and biotic factors influencing the size structure of the aquatic communities in Mediterranean ponds



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DOCTORAL THESIS

Environmental and biotic factors influencing the size structure of the aquatic communities in Mediterranean ponds

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DOCTORAL PROGRAMME IN EXPERIMENTAL SCIENCES AND TECHNOLOGY

SUPERVISED and TUTORISED BY

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“Would you tell me, please, which way I ought to go from here?”
“That depends a good deal on where you want to get to,” said the Cat.
“I don’t much care where—” said Alice.
“Then it doesn’t matter which way you go,” said the Cat.
“—so long as I get somewhere,*” Alice added as an explanation.*
“Oh, you’re sure to do that,” said the Cat, “if you only walk long enough.”

-Alice in Wonderland-

TO MY FAMILY, FOR ALL THE SUPPORT AND
UNCONDITIONAL LOVE

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SUMMARY

Mediterranean ponds are ecosystems with a high ecological value, as they are hotspots for biodiversity and support relevant ecological processes. Nevertheless, they are suffering a strong regression due to anthropogenic pressures, such as agriculture and livestock activities, hydrological modification, habitat destruction, introduction of invasive species, and rainfall reduction because of climate change. Notwithstanding, the received scientific attention has always been far lesser than other larger water bodies. Most ecological studies on Mediterranean ponds have focused on their biodiversity, but only to a lesser extent they have paid attention to the body size structure of their communities. Both taxonomic and size-based approaches should be complementary, as they bring different information to the communities' structure and to the ecological processes. Indeed, body size is related to most physiological and ecological processes (e.g., growth, reproduction, predator-prey interactions, trophic transfer efficiency along the trophic chain) and is also known to respond to environmental changes such as eutrophication, hydrology, and land use modifications, as well as climate warming.

With the purpose of getting a deeper knowledge of the functioning of planktonic communities in Mediterranean ponds and taking the advantage of the very informative size-based approach, the main objective of the present thesis was to analyze which environmental and biotic factors determine the size structure of aquatic communities and populations in different kinds of Mediterranean ponds. With this objective in mind, planktonic communities and populations of the endemic and threatened fish *Aphanius iberus* (Valenciennes, 1846) were analyzed in brackish permanent coastal ponds (Chapter 1 and 2) and in temporary freshwater ponds (except for fish since they are not present; Chapter 3). Size structure was studied using different size metrics (e.g., size diversity index, size spectrum, mean body length, etc.) and applied at different ecological scales (i.e., functional groups, population level, more than one trophic level together). The challenge was to unravel which were the main environmental and biotic drivers shaping the size structure of aquatic communities and populations considering the different ecosystems functioning and different temporal scales at which studies were performed (i.e., spring sampling in

Chapters 1 and 2 and a temporal sampling during three consecutive hydroperiods in Chapter 3). Overall, the key findings can be summarized as follow:

In the **first chapter** the size diversity index of phytoplankton and zooplankton communities in permanent brackish ponds were compared with the respective taxonomic Shannon-Wiener diversity index. According to previous studies, it was hypothesized that the former would be mainly related to biotic interactions that are usually body size dependent (e.g., predation), whereas the latter would be more affected by abiotic variables. Results obtained partially agreed with these predictions, as Shannon-Wiener diversity of both phytoplankton and zooplankton communities was related to pond morphometry, rather than modified by biotic interactions which, instead, affected phytoplankton size diversity. Thus, under food scarcity (higher ratio phytoplankton biomass: total nitrogen, used as a proxy for phytoplankton resource availability), phytoplankton size diversity was lower and small cells dominated the community. Small phytoplankton was likely better competitor under food scarcity due to its low resource requirements and higher efficiency in taking up food resources (higher surface:volume ratio).

In the **second chapter** different size metrics (i.e., size diversity, maximum and mean body length, and body length range) were used as a proxy of the size structure of *Aphanius iberus* populations, an endemic fish inhabiting brackish ponds in the Mediterranean coast of Iberian Peninsula. In general, results showed that a good water quality (evaluated by the biological index QAELS), high water column depth and total nitrogen concentration were related to larger individual sizes and higher size diversity. Water column depth and total nitrogen concentration were also positively related with fish abundances. Therefore, as expected, a good water quality together with a deeper water column (i.e., high habitat availability), would increase the possibilities to find higher densities and well size-structured populations. The positive effect of total nitrogen on the A. *iberus* size structure should be interpreted as an indirect effect, since in the more confined brackish ponds (where total nutrients and organic matter tends to accumulate over years) *A. iberus* populations would have a good status since they are more protected from external perturbations such as freshwater inputs and the presence of freshwater invasive species. On the other hand, *A. iberus* size structure was negatively affected by water conductivity reducing maximum and mean individual length. Although high conductivities prevent the presence of freshwater competitors (e.g., *Gambusia holbrooki*) it may negatively affect *A. iberus* osmoregulatory abilities and its growth performance. Zooplankton biomass (as *A. iberus* food resource) was negatively related with maximum and mean individual length likely due to an increase of consumption rates when increasing fish body size.

In the **third chapter**, the size spectrum was used as a proxy of the size structure of the planktonic community in freshwater temporary ponds. Temporal variations along three consecutives hydroperiods in the phytoplankton and zooplankton' size spectrum parameters (i.e., intercept and slope at both ecological and physiological scaling) as a response to changes in environmental factors and macroinvertebrate predation pressure were analyzed. According to our expectations, size spectrum intercepts at ecological scaling (phytoplankton and zooplankton separately) increased with increasing resource-related variables (e.g., total nutrients, chlorophyll-*a*), reflecting an increase in abundance of individuals in all the size classes. Physiological size spectrum (phytoplankton and zooplankton together) parameters and the zooplankton size spectrum intercept (ecological scaling) increased with decreasing water column depth probably due to a concentration effect of resources and organisms when ponds were drying out. A flattening of the physiological size spectrum slope along the hydroperiod was also observed suggesting an increase of the relative abundance of large zooplankton probably due to an improvement in the energy transfer along the food web as hydroperiod progresses (i.e., sequential development from smaller to larger individuals). Although an increase in the size spectrum slopes (i.e., steeper slopes) and intercepts were expected under high predation pressures, no predation effect was observed, neither zooplankton grazing on phytoplankton nor macroinvertebrate predation on zooplankton. This absence of predation effect on could be explained, respectively, by the presence of colonial phytoplankton (non-edible for zooplankton) and low abundances of macroinvertebrate predators.

RESUM

Les basses mediterrànies són ecosistemes amb un alt valor ecològic, ja que són un "hotspot" de biodiversitat on hi tenen lloc processos ecològics rellevants. No obstant això, estan patint una forta regressió a causa de les pressions antròpiques, com ara les activitats agrícoles i ramaderes, alteracions del règim hídric, la destrucció d'hàbitats, la introducció d'espècies invasores així com la reducció de les pluges a causa del canvi climàtic. No obstant això, l'atenció científica que han rebut aquests ecosistemes sempre ha estat molt menor que la d'altres masses d'aigua més grans. La majoria dels estudis ecològics sobre les basses mediterrànies s'han centrat en la seva biodiversitat, i en canvi, són pocs els estudis que han parat atenció a l'estructura de mides de les seves comunitats. Ambdues aproximacions, la taxonòmica i la basada en les mides corporals dels organismes, haurien de ser complementàries ja que donen informacions diferents sobre l'estructura de les comunitats i els processos ecològics. De fet, la mida del cos d'un organisme està relacionada amb molts dels processos fisiològics i ecològics (p.ex. el creixement, la reproducció, les interaccions depredador-presa, l'eficiència de transferència tròfica al llarg de la cadena tròfica) i se sap que respon a canvis ambientals com l'eutrofització, modificacions del règim hídric i de l'ús del sòl, així com l'escalfament global.

Amb l'objectiu d'aprofundir el coneixement del funcionament de les comunitats planctòniques a les basses mediterrànies, i aprofitant la informació que dona l'enfoc basat en les mides, l'objectiu principal de la present tesi va ser analitzar quins factors ambientals i biòtics determinen l'estructura de mida de les comunitats aquàtiques en diferents tipus de basses mediterrànies. Amb aquest objectiu, es van analitzar les comunitats planctòniques i les poblacions del peix endèmic amenaçat *Aphanius iberus* (Valenciennes, 1846) en basses costaneres salobres permanents (Capítols 1 i 2) i en basses temporànies d'aigua dolça (excepte els peixos; Capítol 3). L'estructura de mides es va estudiar mitjançant diferents mètriques (p.ex., l'índex de diversitat de mides, l'espectre de mides, la longitud corporal mitjana, etc.) i es va aplicar a diferents escales ecològiques (p.ex. grups funcionals, a nivell de població, considerant més d'un nivell tròfic a la vegada). El repte era esbrinar quins eren els principals factors ambientals i biòtics que determinaven l'estructura de mides de les comunitats aquàtiques, tenint

en compte el funcionament dels diferents ecosistemes i les diferents escales temporals utilitzades en aquest estudi (mostreig de primavera als Capítols 1 i 2, i mostreig durant tres hidroperíodes consecutius al Capítol 3). A continuació es resumeixen els resultats més rellevants obtinguts :

En el **primer capítol** es va comparar l'índex de diversitat de mides de les comunitats de fitoplàncton i zooplàncton en llacunes salobres permanents amb el respectiu índex de diversitat taxonòmica de Shannon-Wiener. D'acord amb estudis anteriors, es va plantejar la hipòtesi que el primer índex estaria relacionat principalment amb les interaccions biòtiques que normalment depenen de la mida corporal (p.ex. predació), mentre que el segon índex es veuria més afectat per variables abiòtiques. Els resultats obtinguts van coincidir parcialment amb aquestes prediccions, ja que la diversitat de Shannon-Wiener tant del fitoplàncton com del zooplàncton es va relacionar amb la morfometria de la bassa, en lloc de ser modificada per interaccions biòtiques que, en canvi, van afectar la diversitat de mides del fitoplàncton. Així doncs, en condicions de baixa disponibilitat de recursos alimentaris (reflectida en valors alts de la ràtio biomassa de fitoplàncton: nitrogen total) la diversitat de mides del fitoplàncton va ser menor i les cèl·lules més petites dominaven la comunitat. El fitoplàncton petit seria probablement un millor competidor davant l'escassetat d'aliments a causa dels seus baixos requeriments i una major eficiència en l'ús dels recursos alimentaris (elevada relació superfície: volum).

En el **segon capítol** es van utilitzar diferents mètriques (p.ex., diversitat de mides, longitud corporal màxima i mitjana i rang de longituds corporal) per a estudiar l'estructura de mida de les poblacions d'*Aphanius iberus*, un peix endèmic que habita les basses salobres de la costa mediterrània de la península Ibèrica. En general, els resultats van mostrar que una bona qualitat de l'aigua (avaluada a través de l'índex biològic *QAELS*), una major profunditat de la columna d'aigua i una alta concentració de nitrogen total estaven relacionades amb longituds corporals més grans i elevada una major diversitat de mides. La profunditat de la columna d'aigua i la concentració de nitrogen total també es van relacionar positivament amb l'abundància de peixos. Així doncs, tal com s'esperava, una bona qualitat d'aigua i una major profunditat (més disponibilitat d'hàbitat) afavoririen densitats més elevades i poblacions amb una bona estructura de mides. L'efecte positiu del nitrogen total sobre l'estructura de mides d'*A. iberus* s'ha d'interpretar com un efecte indirecte, ja que a les basses salobres més confinades (on els nutrients totals i la matèria orgànica tendeixen a acumular-se al llarg dels anys) les poblacions d'*A. iberus* estarien en millor estat a l'estar protegides de pertorbacions externes com entrades d'aigua dolça i la presència d'espècies invasores d'aigua dolça. D'altra banda, l'estructura de mides d'*A. iberus* es va veure afectada negativament per la conductivitat de l'aigua que faria disminuir la longitud corporal màxima i mitjana. Tot i que les altes conductivitats impedeixen la presència de competidors d'aigua dolça (p.ex., *Gambusia holbrooki*), aquestes poden afectar negativament les habilitats osmoreguladores d'*A. iberus* i el seu rendiment de creixement. La biomassa del zooplàncton (com a recurs alimentari d'*A. iberus*) es va relacionar negativament amb la longitud corporal màxima i mitjana probablement a causa d'un increment de les taxes de consum a l'augmentar la mida corporal dels peixos.

En el **tercer capítol**, l'espectre de mides es va utilitzar com aproximació de l'estructura de mides de la comunitat planctònica en basses temporànies d'aigua dolça. Es van analitzar les variacions temporals al llarg de tres hidroperíodes consecutius en els paràmetres de l'espectre de mides del fitoplàncton i del zooplàncton (és a dir, la intersecció i el pendent de la recta tant a escala ecològica com fisiològica) com a resposta als canvis ambientals i de la pressió de la depredació per part dels macroinvertebrats. Segons les nostres expectatives, les interseccions de l'espectre de mides a escala ecològica (fitoplàncton i zooplàncton per separat) van augmentar amb l'augment de les variables relacionades amb els recursos (p.ex., nutrients totals, clorofil·la-*a*), reflectint un augment de l'abundància d'individus en totes les classes de mida. Ambdós paràmetres de l'espectre fisiològic (fitoplàncton i zooplàncton junts) i la intersecció de l'espectre de mides del zooplàncton (escala ecològica) van augmentar al disminuir la profunditat de la columna d'aigua, probablement a causa d'un efecte de concentració dels recursos alimentaris i dels mateixos organismes quan les basses s'estaven assecant. També es va observar una disminució del pendent de l'espectre fisiològic al llarg de l'hidroperíode, cosa que suggereix un augment de l'abundància relativa del zooplàncton més gran degut probablement a una millora en la transferència d'energia al llarg de la xarxa tròfica a mesura que avança l'hidroperíode (és a dir, desenvolupament seqüencial d'individus més petits a més grans). Tot i que s'esperava un augment del pendent de l'espectre de mides (pendents més pronunciats) així com de les interseccions en condicions d'elevada pressió de depredació, no es va observar cap efecte de depredació, ni del zooplàncton sobre el fitoplàncton ni dels macro invertebrats sobre el zooplàncton. Aquesta absència d'efecte de depredació es podria explicar, respectivament, per la presència de colònies de fitoplàncton (no comestible per al zooplàncton), i per les baixes abundàncies de macroinvertebrats depredadors.

CONTENTS

Table of Contents

List of Chapters

Chapter 1

Sgarzi, S., Badosa, A., Leiva-Presa, À., Benejam, L., López-Flores, R., & Brucet, S. (2019). Plankton taxonomic and size diversity of Mediterranean brackish ponds in spring: Influence of abiotic and biotic factors. *Water*, *11*(1), 106. **https://doi.org/10.3390/w11010106**

Chapter 2

Sgarzi, S., Brucet, S., Bartrons, M., Arranz, I., Benejam, L., & Badosa, A. (2020). Factors Influencing Abundances and Population Size Structure of the Threatened and Endemic Cyprinodont *Aphanius iberus* in Mediterranean Brackish Ponds. *Water*, *12*(11), 3264.
https://doi.org/10.3390/w12113264

Chapter 3

Sgarzi, S., Badosa, A., Leiva-Presa, A., Bartrons, M., Ersoy, Z., López, E., & Brucet, S. Water depth and resource availability mediate planktonic size spectra in temporary Mediterranean ponds along the hydroperiod. (To be submitted).

List of Figures

List of Tables

1. GENERAL INTRODUCTION

1.1 Mediterranean ponds

Aquatic ecosystems in the Mediterranean region are among the most exploited natural systems in the world (Halpern et al., 2008; Worm et al., 2006), experiencing an estimated 50% loss over the last century (Geijzendorffer et al., 2018; Perennou, C., Beltrame, C., Guelmami, A., Tomas Vives, P. & P., 2012). Despite their important role in providing habitat for animals and plants, diversifying the landscape, or acting as water reserves, they are also very vulnerable habitats, because they are threatened by several anthropogenic pressures (Céréghino et al., 2008; Oertli et al., 2005) such as hydrological modification, habitat destruction, or the introduction of exotic species (Doadrio, 2001; Vilà & García-Berthou, 2010). Moreover, aquatic ecosystems in Mediterranean area may be among the most impacted by climate change since Mediterranean region is recognized as climate change Hot-Spot. Indeed, in the Mediterranean basin some climate changes have already started to happen, such as an increase in annual mean temperatures (Cramer et al., 2018) and a rise in the frequency of drought events due to severe reduction in rainfall. All these changes are expected to affect the characteristics of aquatic ecosystems in this area such as changes in hydrology, water chemistry, and sediment loads (Fischlin & Midgeley, 2007; Mariotti et al., 2008; Pearce & Crivelli, 1994), directly affecting their biodiversity.

Among Mediterranean aquatic ecosystems, ponds are ecologically very important ecosystems that, despite their small size, support relevant hydrological, chemical, and biological processes. They are also biodiversity hotspots in terms of both species' composition and biological traits (Céréghino et al., 2014; Williams et al., 2004). Moreover, Mediterranean ponds provide important ecosystem services, such as the retention of nutrients and hydrological regulation (Céréghino et al., 2008), the improvement of water quality, the sequestration of CO_2 from the atmosphere (Zedler & Kercher, 2005), in addition to their aesthetic and landscape value. They are characterized by a hydrological regime strongly conditioned by the Mediterranean climate, which is characterized by the unpredictability and irregularity in water inputs, which mainly occur in autumn-winter. Summers are characterized by hot and dry weather, whereas winters are usually mild and wet. This characteristic hydrology is a determinant of the ecological functioning of those ecosystems, leading to important fluctuations in their physical, chemical, and biological composition (Beklioglu et al., 2003; Boix et al., 2004; López-Flores et al., 2003; Quintana et al., 1998). Depending on different characteristics, such as phreatic influence, external water inputs (freshwater or salty), basin depth and proximity to the sea, Mediterranean ponds include from permanent to temporary and from freshwater to brackish and hypersaline ecosystems. Due to the capacity of Mediterranean ponds to respond to long-term disturbance of global change, compared to bigger aquatic ecosystems, they are considered an important warning system (Céréghino et al., 2008; De Meester et al., 2005; Zacharias & Zamparas, 2010).

1.2 Brackish coastal ponds

Mediterranean brackish coastal ponds are characterized by shallow waters, limited surfaces, and rapid and wide changes of physical-chemical (e.g., salinity and nutrients) and biological parameters due to natural events (e.g., seawater inputs, intense rainfalls) or to anthropogenic alterations (e.g., agricultural runoff) (Fois et al., 2021). They are also exposed to a variable degree of confinement (i.e., prolonged absence of water inputs), depending on the connections with sea, freshwater inputs, and evaporation rates (McLusky & Elliott, 2007; Quintana et al., 2004). As a general pattern, oxidized nitrogen compounds (i.e., nitrates, nitrites) increases during flooding events, whilst low water level periods (e.g., during confinement) favour the accumulation of organic matter, total nitrogen and phosphorus, and the loss of the inorganic forms of nitrogen due to denitrification (Badosa et al., 2006). Salinity also increases in periods of drought due to evaporation. Nevertheless, factors such as human regulation of freshwater and riverine inputs (e.g., agricultural activities), and the direct connection with the sea (Comin & Valiela, 1993; Frascari et al., 2002; Villena & Romo, 2003) can alter this nutrient's dynamics and the structure of aquatic communities.

In Mediterranean coastal ponds, salinity or conductivity levels, water turnover and productivity are described as the main factors determining species composition of aquatic communities, which have a high interannual variability (Alvarez Cobelas et al., 2005; Britton & Crivelli, 1993). In fact, brackish and hypersaline ponds are characterized by low species richness and diversity (Brucet et al., 2012; Green et al., 2005; Sosa-López et al., 2007) with the frequent dominance of a single species (Brucet, Boix, et al., 2005). Communities need to be well adapted and tolerant to high salinity levels (Gascón, Boix, Sala, et al., 2009; Waterkeyn et al., 2008), which sometimes are even higher than sea water due to marine intrusion and evaporation. For the communities which live in these harsh environments, salinity plays a very important role in shaping species composition and food web interactions (Brucet et al., 2009, 2010). For instance, zooplankton has been observed to experience a shift from larger species (e.g., cladoceran), to smaller individuals such as copepods, small cladoceran and rotifers along the salinity gradient (Brucet et al., 2010). Increasing conductivity is usually related with a reduction in species richness (Brucet et al., 2009; Cognetti & Maltagliati, 2000; Moss, 1994), because many species have a low osmoregulatory ability. Phytoplankton must also adapt to frequent hydrological perturbations affecting salinity levels, such as pond isolation in summer and sea storms (López-Flores, Garcés, et al., 2006), which, together with nutrients availability, are considered the most important factors determining phytoplankton composition and distribution (Comin & Valiela, 1993; López-Flores, Garcés, et al., 2006; Specchiulli et al., 2008).

On the other hand, fish predation pressure may also be a driver of zooplankton community structure in brackish coastal ponds (Badosa et al., 2007; Brucet, Quintana, et al., 2005; Quintana et al., 2006) and, indirectly, of phytoplankton community structure (Compte et al., 2012) (see Figure 1). In the Mediterranean coast of Iberian Peninsula, brackish permanent ponds are the habitat for the cyprinodont 'Spanish toothcarp' (*Aphanius iberus*, Valenciennes, 1846), a small euryhaline endemic fish critically threatened by habitat destruction, intensive agricultural activities, and the introduction of exotic fish species, such as *Gambusia holbrooki*, which is known to share similar habitat (Vargas *et al*., 1993) and food resources (Alcaraz & García-Berthou, 2007; Haiahem et al., 2017). *A. iberus'* diet is mainly composed of copepods adults and nauplii, and detritus (Alcaraz & García-Berthou, 2007). Regarding the habitat of *A. iberus*, it previously included a wide range of lowland waterbodies, but now it is restricted to brackish and hypersaline coastal waterbodies (Doadrio, 2001; Oliva-Paterna et al., 2006) since its main competitors are less salt-tolerant (Leonardos & Sinis, 1998). In some brackish coastal ponds, jellyfish (e.g., *Odessia maeotica*) and, at a lesser extent *Gammarus aequicauda* can also act as planktoninc trop predators causing drastic changes in the zooplankton community structure (Compte et al., 2010, 2012).

1.3 Freshwater temporary ponds

In the Mediterranean region, temporary aquatic habitats represent the great majority of freshwater ecosystems (Blondel & Aronson, 1999). Temporary ponds are shallow ponds that undergo a periodic cycle of flooding and drought, whose duration depends on the weather. The flooding phase usually coincides with autumn or spring rainfalls (depending on annual variations) whereas drought usually occurs in summer (Dimitriou et al., 2006; Ghosn et al., 2010; Zacharias et al., 2008). Nevertheless, some temporary ponds can hold water for more than one year, whereas others for only few weeks. The length of the hydroperiod is the main determinant of faunal composition and of the structure of communities (Boix et al., 2001). In fact, species richness normally increases as the length of the flooded period increases (Beja & Alcazar, 2003; Blaustein & Schwartz, 2001), along with competition for resources. The hydroperiod length, together with the origin of water (i.e., rain-fed or karstic ponds), are considered the main drivers of the nutrients' dynamics in temporary ponds (Chaichana et al., 2011; Davies et al., 2008; Declerck et al., 2006). In fact, the shorter is the hydroperiod, the lower are total nitrogen concentration, the planktonic chlorophyll-*a* and the organic matter accumulated in the sediment due to the low time available for plant development (Sahuquillo et al., 2012). At the same time, shorter hydroperiods entail higher turbidity and higher total phosphorous concentration in water due to resuspension driven by wind or, in some cases, trampling by livestock (Declerck et al., 2006; Søndergaard et al., 1992). This low Total nitrogen/ Total phosphorous ratio in temporary ponds (especially in those with shorter hydroperiods), is explained because desiccation favours mineralization of organic matter, corresponding to a loss of N in gas form from the sediment (Busnardo et al., 1992; Fernández-Aláez & Fernández-Aláez, 2010). Thus, disrupting flooding enhance nitrogen loss.

As temporal variability in water composition favours large environmental gradients over short time and spatial scales, temporary ponds support many endemic and rare species, although this biodiversity is often neglected (Boix et al., 2001). Biodiversity at a regional scale is generally very high but at a local level temporary ponds tend to have lower species richness, but more uncommon and rare species, than permanent ponds since they need to deal with desiccation (Céréghino et al., 2008). Communities inhabiting these ephemeral systems depend greatly on the hydrological changes, as many species synchronize their reproductive cycle depending on the flooding duration (Zacharias & Zamparas, 2010). The hydrological regime's alteration may lead to a decline of those adapted species, also modifying the biotic interactions. In fact, because of climate change (i.e., reduced precipitation and extended drought)

(Calhoun et al., 2017), Mediterranean temporary ponds could experiment extreme shifts in aquatic invertebrates' diversity and in plant species composition (Bagella et al., 2013; Ghosn et al., 2010), in comparison to ponds in boreal latitudes.

In absence of fish, in temporary ponds macroinvertebrates act as top predators (e.g., Odonata, Coleoptera among the most voracious) of zooplankton, becoming more relevant in determining the trophic structure of the aquatic community (Wellborn et al., 1996) (Figure 1). During the flooding phase trophic resources become available, and competition and predation are low. During the drying out environmental parameters change, and higher density of fauna leads to a greater competition and predation. (Boix et al., 2004; Lake et al., 1989; Spencer et al., 1999). The longer is the hydroperiod, the stronger is the effect of such biotic interactions (Schneider & Frost, 1996; Wellborn et al., 1996). Thus, the hydroperiod length and the flood regime are the main factors explaining the species composition of aquatic communities (Sim et al., 2013; Williams, 2006), as higher species richness is associated to a longer hydroperiod (Blaustein & Schwartz, 2001; Schneider & Frost, 1996). Studies on the phytoplankton community of temporary ponds are scarce and some suggest that temperature and precipitation patterns determine at a good extent the structure and dynamics of phytoplankton assemblages at a regional scale (Barone et al., 2010; Naselli-Flores & Barone, 2002).

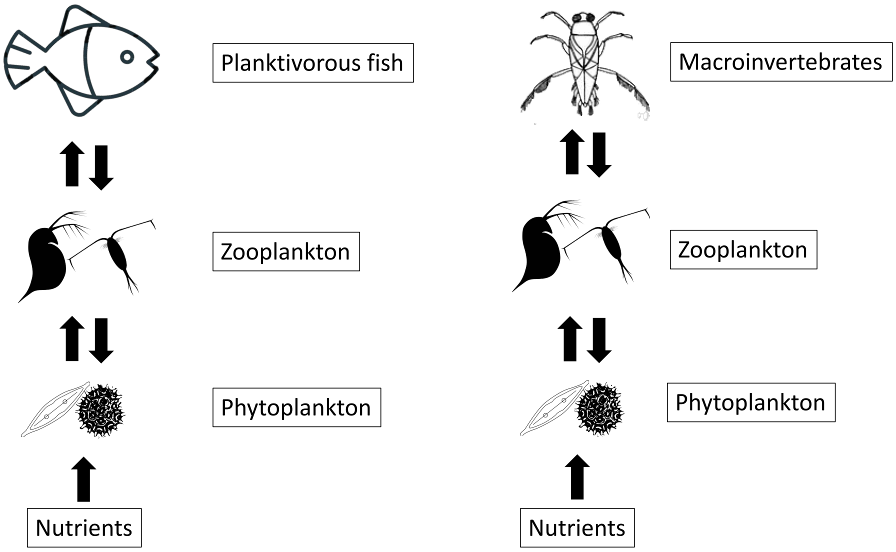


Figure. 1 Example of two simplified trophic chains in Mediterranean permanent (on the left) and temporary (on the right) ponds. From the bottom, nutrients are up taken by the first trophic level, composed by phytoplankton (i.e., primary producers). The second level is composed by zooplankton (i.e., primary consumers), whereas the secondary consumers levels are represented by different organisms: planktivorous fish for permanent ponds, and macroinvertebrates for temporary ponds. The arrows represent trophic interactions among the levels. The upwards arrows show bottom-up, whereas downwards arrows represent top-down control.

Due to their ecological importance and biodiversity, Mediterranean temporary ponds are considered habitats of priority interest by the Habitats Directive (Council Directive 92/43/EEC) of the European Union and are part of the Natura 2000 Network. Nevertheless, temporary ponds were excluded from the European Water Framework Directive that focused mainly on waterbodies greater than 50ha (Kristensen & Globevnik, 2014; Van Den Broeck et al., 2015). As agriculture and urban development play an increasing role in the Mediterranean region, temporary ponds are in danger of degradation (Beja & Alcazar, 2003; Blaustein & Schwartz, 2001; Brendonck & Williams, 2000). Those pressures are found to affect mainly the water quality and the ecological status of those ecosystems (Zacharias et al., 2008). However, temporary aquatic environments have been studied far less frequently than permanent ones (Williams, 2006).

1.4 Structure of aquatic communities: size-based approach

Most ecological studies of Mediterranean ponds have been focused on their biodiversity (Della Bella et al., 2005; García-Girón et al., 2019; Lumbreras et al., 2016), the dynamics of populations and communities (Anton-Pardo & Armengol, 2012; Boix et al., 2004; Brucet et al., 2012; Ruhí et al., 2009), and their relationship with nutrients variability and hydrological patterns (Àvila et al., 2018; Badosa et al., 2006; Brucet, Boix, et al., 2005; Gascón et al., 2005), as well as with anthropogenic pressures (Beja & Alcazar, 2003; Gascón et al., 2012). Although the taxonomic approach is very important to understand the community structure, it considers species identity rather than their functional roles (e.g., trophic interactions). Community structure can also be studied from a size-based point of view which have been found to give complementary information to the taxonomic approach (Rodriguez & Magnan, 1993; Strayer, 1991). Indeed, most ecological and physiological processes such as growth, reproduction and population growth rates are body-size dependent (Brown et al., 2004; Peters & Wassenberg, 1983). Even though body size is an attribute of individual organisms, it can also be applied at higher levels of ecological organization (Cohen et al., 1993; Cousins, 1980; Emmerson & Raffaelli, 2004), making it a good candidate for answering many ecological questions and explaining general patterns in natural systems (Peters & Wassenberg, 1983).

Notwithstanding the great relevance of size-approach in ponds ecosystems, the great majority of studies considering body size as a community organizing unit focus on marine and lake systems (Kerr & Dickie, 2001; Platt & Denman, 1977). Harsh environmental conditions in Mediterranean ponds make size-based interactions (i.e., predation and competition) even more relevant than in other ecosystems (Gascón, Boix, Sala, et al., 2009). For example, a classical taxonomic approach does not consider that different developmental stages of the same species could occupy different trophic niches, feeding on different preys, to avoid intraspecific competition under low resource availability (Brucet et al., 2006, 2008).

In this sense, precious information about predator-prey interactions, competition, population dynamics, nutrient cycling, and trophic transfer efficiency along the trophic chain can be given by the body size structure (Ersoy et al., 2017; Gaedke, 1995; Woodward et al., 2005; Yvon-Durocher & Allen, 2012). In fact, in zooplankton communities, competition for resources is typically body-size dependent as the largest species are usually better competitors than the smallest under food limitation (e.g. size efficiency hypothesis in zooplankton; (Declerck et al., 1997; Dodson & Brooks, 1965; Kreutzer & Lampert, 1999)). As planktivorous fish predation is also size-selective (Brucet et al., 2010; Dodson & Brooks, 1965), larger zooplankton is removed by fish, allowing the dominance of the smallest organisms (Dodson & Brooks, 1965; Hall et al., 1976; Vanni, 1986). Indeed, several studies have shown that fish predation is the most important driver of zooplankton size structure, as an increase in fish density is related to a decrease in density, mean body size and size diversity of zooplankton in addition to changes in its taxonomic composition (Brucet et al., 2010; Finlay et al., 2007; Jeppesen et al., 1997; Zimmer et al., 2001). When planktivorous fish are reduced or absent, such as in temporary ponds, macroinvertebrate predation may also affect zooplankton species composition and size distribution (Hampton et al., 2000; Hampton & Gilbert, 2001; Wellborn et al., 1996). In fact, the most voracious insects (e.g., notonectids) increasingly ignore the small preys as they grow larger (Cooper, 1983; Scott & Murdoch, 1983), showing a size-selective predation. In phytoplankton communities, body size is also important in nutrient acquisition at low concentrations since small individuals are more efficient than the large ones due to their low resource requirements and high cell's surface-to-volume ratio (Litchman & Klausmeier, 2008). Concerning phytoplankton, recent studies have shown that its size structure, analysed through the size diversity index (see 'Size diversity index' section 1.4.1), is mainly determined by changes in resource availability, rather than by predation, due to the prevailing influence of abiotic factors in their nutrient uptake (Brucet et al., 2017; Ersoy et al., 2017; Marañón et al., 2015; Quintana et al., 2014).

Since trophic interactions within the food web are mainly size-dependent, size structure within a trophic group of the planktonic food web can be determined by the size structure of the adjacent trophic levels (Boix et al., 2004; Boudreau & Dickie, 1992; Brucet, Boix, et al., 2005; Dickie, 1987; Quintana et al., 2006). It is worth to mention that size-based interactions are particularly relevant in the species-poor communities like those of Mediterranean brackish ponds, where trophic interactions are mainly body-size dependent (Badosa et al., 2007; Quintana et al., 2006). Moreover, body size is also known to respond to changes in the environment such as eutrophication, hydrological events, land use and climate warming (Ahrens & Peters, 1991; Brucet, Boix, et al., 2005; Sprules & Munawar, 1986; Zhang et al., 2013). Therefore, size-based approach is considered effective to understand the functioning of aquatic communities and might be useful to evaluate size-based interactions such as competition and predation, providing important insights into the Mediterranean pond ecology.

Size structure can be studied at different ecological scales: at level of functional group such as phytoplankton, zooplankton and fish (e.g., Chapter 1 of this thesis) (Brucet et al., 2010; Dossena et al., 2012; Emmrich et al., 2014), at population level (e.g., Chapter 2 of this thesis) (Oliva-Paterna et al., 2009; Vila-Gispert & Moreno-Amich, 2001), or integrating different trophic levels (e.g., Chapter 3 of this thesis) (Brucet, Boix, et al., 2005; Quintana et al., 2002). The size structure of aquatic communities can be analysed through different methodologies such as mean size, maximum size, and size range (e.g., Chapter 2 in this thesis), calculating the size diversity index (e.g., Chapter 1 and 2 in this thesis) or modelling the size spectrum (e.g., Chapter 3 in this thesis).

1.4.1 Size diversity index

The size diversity index (μ) indicates how the body sizes and number of individuals are distributed along the size structure. It is computed based on the Shannon diversity expression adapted for a continuous variable (Pielou, 1969), such as body size, and it produces values in a similar range to those of the Shannon species diversity index (Brucet et al., 2006, 2010; Quintana et al., 2008). This measure takes the form of an integral involving the probability density function of the size of the individuals described by the following equation:

$$\mu(x) = -\int_{0}^{\infty} \rho_x(x) log_2\, \rho_x(x) dx$$

where ρ_x(x) is the probability density function of size x. The nonparametric Kernel estimation is used as a probability density function (Quintana et al., 2008, 2016), which is applicable to any type of size distribution. A low size diversity represents a narrow size range with an uneven distribution of the abundances among the size classes, wheras a high value of size diversity indicates a wide size range and/or even representation of the different sizes along the size distribution (Figure 2) (Emmrich et al., 2011). As it gives a unique value per size distribution, the principal advantage is that it avoids the arbitrariness introduced when using size classes as done in size spectrum models (see next section) and has an intuitive interpretation of its ecological meaning (Quintana et al., 2008), as the concept of diversity is well established. Size diversity provides complementary information about the community structure to classical taxonomical approaches (Brucet et al., 2006, 2008; Ersoy et al., 2017; Quintana et al., 2006). For example, some studies have shown that size diversity may reflect better the biotic interactions (e.g., predation or inter-and/or intraspecific competition) than taxonomic diversity (Badosa et al., 2007; Gascón, Boix, Sala, et al., 2009).

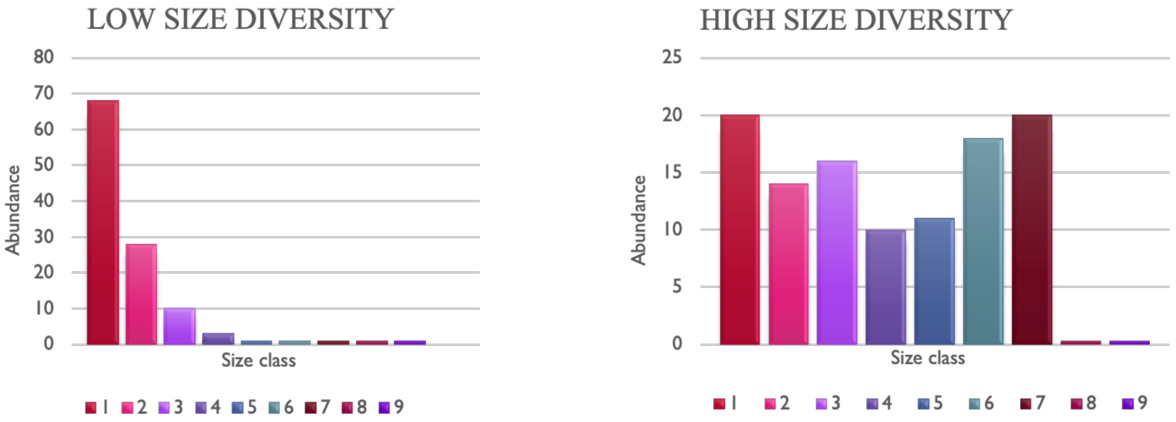


Figure 2. Conceptual examples of low (on the left) and high (on the right) size diversity. On the Y-axis is represented the overall abundance out of 100%, whereas on the X-axis different size classes are shown.

1.4.2 Size spectrum

One of the methodologies more often applied to explain the size structure of a diverse range of aquatic communities (Yvon-Durocher et al., 2011) is the normalized biomass-size spectrum (NBSS) (Platt & Denman, 1977). It is based on the biomass-spectrum or "Sheldon spectrum" (Sheldon et al., 1972), firstly applied in marine studies for planktonic particles. Following the binning method (Sprules & Barth, 2016; White, 2008), individual sizes are grouped into different size classes in log scale. The NBSS improved the Sheldon spectrum by dividing biomass in each size interval by the width of the size interval, because the width of the logarithmic size class intervals increases as body size classes increase (Sprules & Barth, 2016). The NBSS shows the inverse relationship that exists between the abundance of organisms and their size (Figure 3). In other words, organism abundance is inversely proportional to size (at double logarithmic scale) because of the faster metabolism of small-sized organisms and the loss of energy to the upper levels of the trophic chain (Platt & Denman, 1977; Sprules & Munawar, 1986). Both the normalized biomass-size spectrum (NBSS) and the normalized abundance-size spectrum (NAS; using organisms' abundance instead of biomass) give a linear relationship between normalized biomass and normalized numerical abundance of individuals, respectively, and size classes (White, 2008) with a theoretical value for the slope across ecosystems and organisms at steady state conditions. The former has usually a theoretical slope close to -1, whilst the latter has theoretical slope closed to -2 (Polishchuk & Blanchard, 2019). Size spectrum can be constructed considering a single community or trophic level (i.e., ecological size spectrum; (Brucet, Boix, et al., 2005; Dickie, 1987), or the whole food web (i.e., physiological spectrum; (Sprules & Barth, 2016). Whereas the ecological spectrum is more related to biotic interactions within a trophic level such as predation and competition, the physiological spectrum reflects the TTE (trophic transfer efficiency or the ratio of production rates between adjacent trophic levels) of the food web (Brown et al., 2004; Mehner et al., 2018; Treblico et al., 2013).

Changes in the size spectrum slope have been reported in aquatic ecosystems as a response to environmental changes, human impacts, and predation (Blumenshine et al., 2000; Gaedke, 1992; Quintana et al., 2002; Zimmer et al., 2001). Compared to the theoretical slope, steeper slopes (i.e., more negative slopes) represent an increase in the relative abundance of smaller individuals (Figure 3). On the contrary, flatter slopes (i.e., less negative slopes) mean an increase in the relative abundance of larger individuals and the simultaneous decrease of the relative abundance of smaller ones (Figure 3). Many studies observed flatter size spectrum slopes (i.e., large individuals are favoured) as a response to increase nutrient availability (Ahrens & Peters, 1991; Quintana et al., 2002; Sprules & Munawar, 1986), but steeper slopes (Yvon-Durocher et al., 2011) after an increase in temperatures, which seems to favour smaller body sizes (Atkinson et al., 2003; Gardner et al., 2011; Havens et al., 2015). Biotic factors such as predation has been found to cause a decrease in relative abundance of larger organisms resulting in a steeper size spectrum slope (Almond et al., 1996; Brucet, Boix, et al., 2005).

The size spectrum intercept is a gross estimator of the food-web capacity or productivity potential (i.e., biomass or number of individuals supported by the food web; (Gaedke, 1992; Sprules & Munawar, 1986) (Figure 3). Similarly, as for the slope, changes in the intercept have been related to changes in environmental conditions (Ahrens & Peters, 1991; Sprules & Barth, 2016; Sprules & Munawar, 1986; Zhang et al., 2013) such an increase in productivity, temperature, and nutrient availability (Ahrens & Peters, 1991; Benejam et al., 2018; Sprules & Barth, 2016; Sprules & Munawar, 1986). Conversely, an increase of predation interactions may induce a drop in the size spectrum intercept (i.e., lower abundances, (Murry & Farrell, 2014; Shin et al., 2005; Zimmer et al., 2001)).

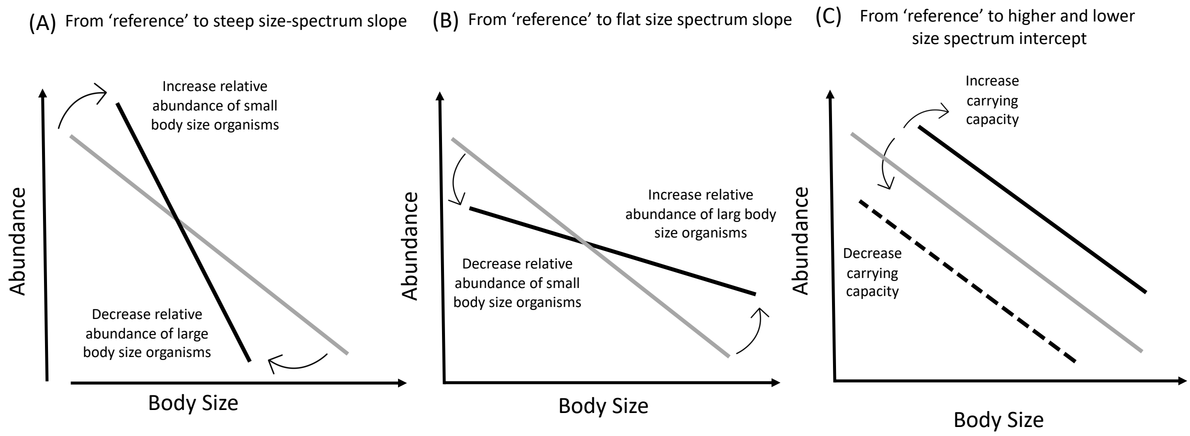


Figure. 3 Adapted from Arranz *et al.*, 2021. Hypothetical changes in size spectrum parameters (slope, A and B and intercept, C). In each panel, the X-axis represents organisms' body size and the Y-axis their numerical abundance, both in log2-log2 space. The black line represents the hypothetical change of the size spectrum slope and intercept, whereas the grey line represents the "reference" size spectrum. Panel A depicts the hypothetical increase of relative abundance of small organisms and decrease of relative abundance of large organisms. Panel B depicts the hypothetical relative increase in large organisms and decrease of relative abundance of small organisms. Panel C depicts the hypothetical changes of the size spectrum intercept (measured as the carrying capacity of the assemblage) as complementary information to interpret changes on the size spectrum slopes. The dash line indicates low carrying capacity whereas the solid black line indicates an increase of the carrying capacity compared to the reference size spectrum.

2. GENERAL OBJECTIVE

The principal objective of this thesis is to explore the biotic and abiotic variables influencing the size structure of the planktonic community in Mediterranean ponds (including freshwater, brackish, permanent, and temporary ponds). The size structure is studied for different trophic levels (phytoplankton and zooplankton in chapter 1 and 3 and fish in chapter 2) and at different scales (population size structure, community size structure, and trophic web size structure). I also analysed the size structure at different temporal scales (a snapshot sampling in chapter 1 and 2 and a monthly sampling during 3 years in chapter 3). Finally, I employed different methods to analyse the size structure: (1) the size diversity index (chapter 1), (2) several size-metrics (e.g., mean and maximum body size and body size range) together with the size diversity index (chapter 2), and (3) the abundance-size spectrum and its parameters (slope and intercept) (in chapter 3).

2.1 SPECIFIC OBJECTIVES

2.1.1 Chapter 1:

The objective of this chapter was to determine the effects of biotic interactions and abiotic factors on the size structure of phytoplankton and zooplankton in Mediterranean brackish permanent ponds during spring season. In addition, I aimed to explore if the size structure and taxonomic structure were affected by the same drivers including predation, food resource availability, and environmental variables.

2.1.2 Chapter 2:

In this chapter the focus was on the size structure of the populations of the endemic cyprinodont fish *Aphanius iberus* in Mediterranean brackish permanent ponds during spring season. The aim was to assess whether environmental factors (e.g., conductivity, nutrients, pond morphology), the water quality and food resource (i.e., zooplankton biomass) determine population abundance and size structure, which was analysed through several size-metrics (i.e., mean, maximum size and size range) and the size diversity index. Another aim was to test if the presence of the main competitor of *A. iberus, Gambusia holbrooki*, was decisive in determining changes the size structure the of the endemic fish.

2.1.3 Chapter 3:

The last chapter aimed to model the size spectrum of phytoplankton and zooplankton communities though time (i.e., hydroperiod gradient) in Mediterranean freshwater temporary ponds, and to analyse the influence of environmental variables (e.g., temperature, nutrients, conductivity, water column depth) and predation (as a biotic interaction) on the size spectrum parameters (i.e., slope and intercept) at community and trophic web level (i.e., ecological and physiological scale, respectively).
A summary of the specific objectives can be found at Figure 4.

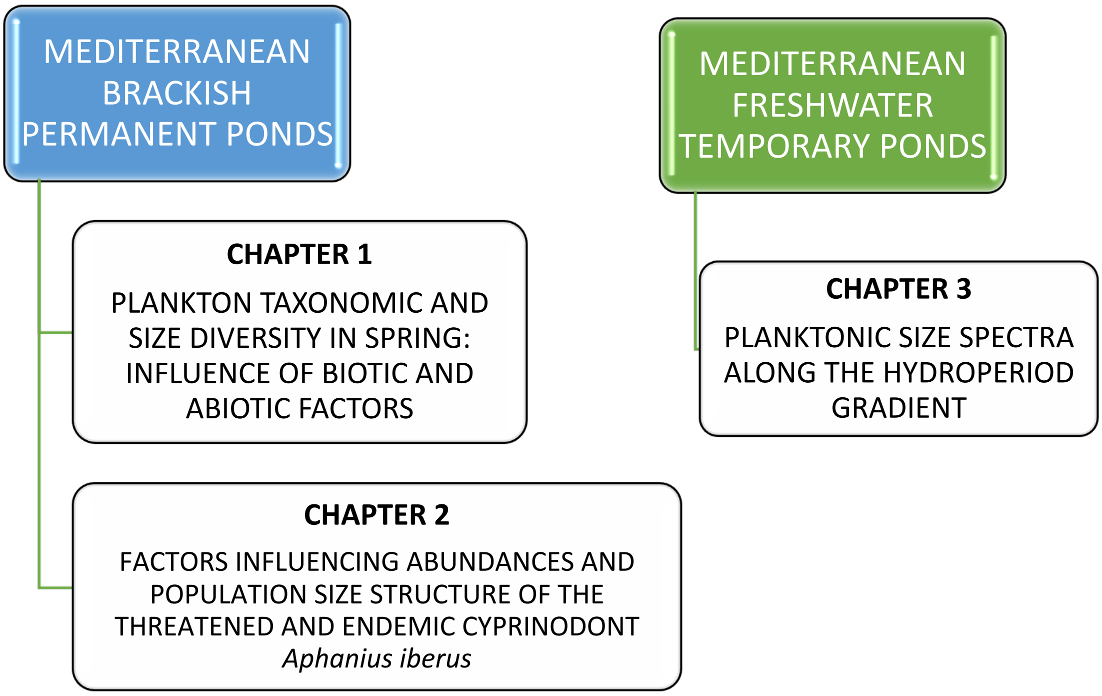


Figure 4. Main objectives of the chapters of the present thesis separated by the type of ecosystem.

3. METHODOLOGY

3.1 Study area

The study area was located in the ‘Empordà’ area, in Catalonia, (NE Spain) and included a total of 16 Mediterranean ponds, 13 of which were brackish permanent ponds (included in Chapters 1 and 2) and three were freshwater temporary ponds (included in Chapter 3) (Photo S1, Supporting Information). Brackish ponds were located in two protected coastal areas situated between 42°01′42″ N–3°11′18″ E and 42°15′58″ N–3°08′17” E. Ten of these ponds were located in the “Aiguamolls de l’Empordà” Natural Park (Figure 5, 2), and the other three were located south in the “El Montgrí, Illes Medes i el Baix Ter” Natural Park (Figure 5, 3). Freshwater temporary ponds were placed in the Can Torres estate in the ‘Albera’ Mountain Range, a protected area located between 42°23′52″ N–2°58′52″ E, (Figure 5, 1).

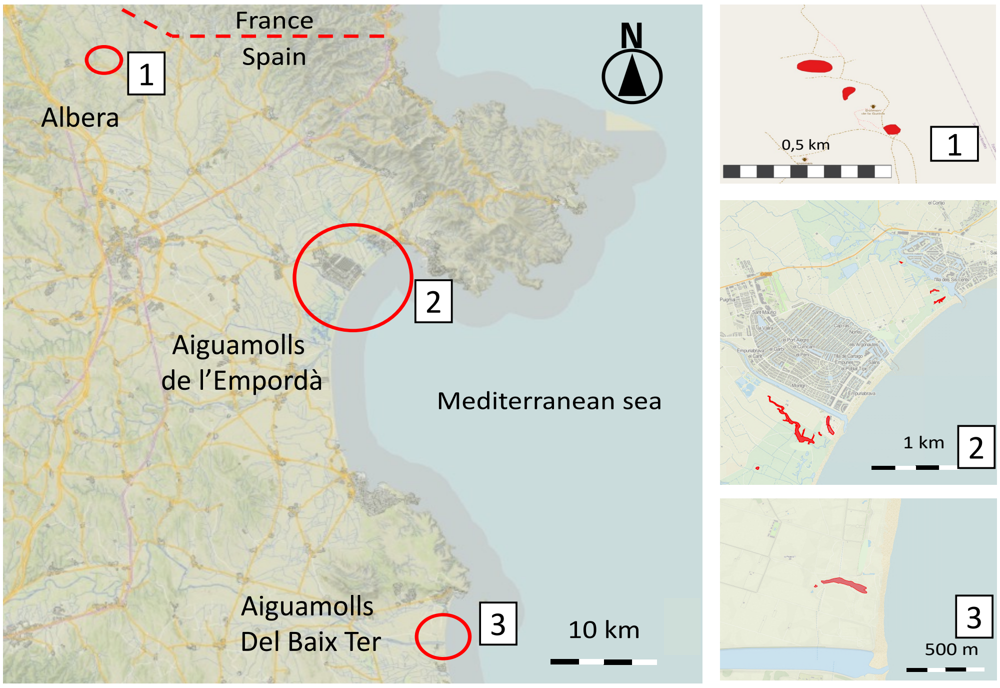


Figure 5. General map of the location of the study area, with a zoom on the three locations that host the studied ponds (in red colour in the maps on the right): map number 1 shows the three freshwater temporary ponds of “Albera”, whereas maps 2 and 3 show the 13 brackish permanent ponds of the Natural Parks “Aiguamolls de l’Empordà” and “El Montgrí, Illes Medes i el Baix Ter”, respectively. This map was produced with the online software **QGIS** Development Team (3.18.3. ‘Zürich’ 2021). **QGIS** Geographic Information System.

All the ponds of the present thesis are characterized by Mediterranean climate with hot, dry summers and mild, wet winters (Quintana et al., 2006). Brackish coastal ponds have a hydrological pattern mainly determined by long periods of drought (spring and summer), when the water level gradually decreases, which are irregularly interrupted by sudden flooding events such as seawater inputs during sea storms or intense rainfalls (autumn-winter), (Badosa et al., 2006; Quintana, 2002; Quintana et al., 2004). Sometimes, during drought, ponds' water can reach very high conductivities, becoming hypersaline. Salinity and nutrient dynamics in these ecosystems are strongly affected by this hydrology. In contrast to many temperate lakes, in these confined Mediterranean ponds nitrogen rather than phosphorus is usually the limiting nutrient for primary production (e.g., (Badosa et al., 2007; López-Flores et al., 2009; Quintana et al., 2004)) due to strong denitrification processes, low water turnover and the high internal load of phosphorous (Comin & Valiela, 1993; Quintana et al., 1998).

The studied brackish ponds are characterized by shallowness (max. water column depth of 150cm), different surfaces areas ranging from 14.9 m^2 to 68150.0 m^2, and conductivities ranging from 10.07 $mS \cdot cm^{-1}$ to 69.10 $mS \cdot cm^{-1}$ (see Table 1). Similarly, temporary ponds usually start the flooding process with the late-autumn or spring rainfalls to dry out in summer (Ruhí et al., 2014). The length of these periodic cycles of flooding and drought depend mainly on precipitations and external temperatures, as groundwater inputs are absent and they are isolated from other water bodies (Ruhí et al., 2014). Temporary ponds are also shallow (max. water column depth of 61.2 cm) with areas ranging from 1626.0 m^2 to 4977.5 m^2 and showing lower conductivities (from 0.12 to 0.22 $mS \cdot cm^{-1}$) than brackish ponds (Table 1).

In brackish ponds, during confinement, some persistent pesticides and heavy metals tend also to concentrate (López-Flores et al., 2003). In fact, although laying in two Natural Parks, there are multiple stressors that reduce the conservation status and the water quality of these brackish ecosystems. Both Natural Parks are in coastal areas, exposed to high anthropogenic pressures such as intense urbanization, massive tourism, and the introduction of invasive species (Carmona-Catot et al., 2013; Salvadó et al., 2006). Indeed, "El Montgrí, Illes Medes i el Baix Ter", where three of the studied ponds lay, only recently (year 2010) has been declared a Natural Park. "Aiguamolls de l'Empordà" Natural Park was created in 1983. Thanks to 'S.O.S. fartet' project (fartet is the common Catalan name of *Aphanius iberus*), supported by 'Fundación Biodiversidad' of the 'Ministerio para la transición ecologica y el reto demográfico' of Spanish Government, and carried out in both Natural Parks in 2016 to study the conservation status of *Aphanius iberus*, the data for the first and second chapters were obtained.

The anthropogenic disturbances acting on the coastal area are negligible in the ‘Albera Mountain Range’ where the three studied temporary ponds lay. The Albera region is not a Natural Park, but it is considered a natural reserve, as it is a protected space of ‘Natura 2000 network’. The studied ponds are located in the inland region of Albera that is a mountain range characterized by hay meadows and wetlands of high floristic and faunistic interest. In fact, urbanization is very limited, and the number of visitors is very reduced, resulting in a lower touristic pressure. The data of the third chapter were obtained from the project: ‘Seguiment, gestió i recuperació de basses temporànies i prat de dall a la finca vitivinícola de Can Torres (serra de l’Albera, Alt Empordà)’, whose translation would be: ‘Monitoring, management and recovery of temporary ponds and mowing meadows on the Can Torres wine estate (Albera Mountain Range), which aimed to monitor the environmental characteristics and aquatic communities of one restored pond, and compared them to that of pre-existing natural temporary ponds. This project was funded by the private ‘ANDRENA’ foundation (Register of Private Foundations of Generalitat de Catalunya no. 2978).

Because of the different anthropogenic pressures to which the studied areas (coastal and inland) are subjected, the studied ponds showed very different ecological status and water qualities according respectively to the indexes *ECELS* (the acronym refers to the Catalan: ‘Estat de Conservació d’Ecosistèmes Lenítics Soms’, or Shallow Lentic Ecosystems Conservation Status), and *QAELS* (acronym of the Catalan: ‘Qualitat de l’Aigua d’Ecosistèmes Lenítics Soms’ or Water Quality of Lentic Shallow Ecosystem). The former is based on morphological aspects, type of aquatic vegetation and human impacts (Sala et al., 2004), and the latter is based on the aquatic invertebrate community (Boix et al., 2005; Quintana, Cañedo-Argüelles, et al., 2015). Whereas brackish coastal ponds sampled during spring showed values of *ECELS* index ranging from ‘deficient’ ($30 \leq$ *ECELS* ≤ 50) to ‘very good’ ($90 \leq$ *ECELS* ≤ 100), and *QAELS* indexes ranging from ‘bad’ (*QAELS*<0.46) to ‘mediocre’ ($0.55 \leq$*QAELS*<0.62), in temporary freshwater ponds a ‘very good’ ecological status ($90 \leq$ *ECELS* ≤ 100) and ‘good’ water quality ($0.58 \leq$*QAELS*<0.86) were observed (Table 1).

Table 1. Main morphometric characteristics of the studied ponds, along with the geographic coordinates (Latitude and Longitude), conductivity values (EC), and the *ECELS* and *QAELS* indexes values of ecological status and water quality, respectively. Loc stays for 'Location'. It shows in which area the ponds lay: 'AE' are the 'Aiguamolls of Empordà', Natural Park, 'MIMBT' are the 'El Montgrí, Illes Medes i el Baix Ter' Natural Park, and 'ALB' stays for the protected area of 'ALBERA'. 'Chapter' shows which pond was considered in each chapter of the thesis. It is important to notice that the 'Depth' (Mean water column depth), 'Area' (Pond area) as well as 'EC' (conductivity) and *QAELS* index for the freshwater temporary ponds (Rajoleria, Cardonera, Prats del Roser) are the results of the average values along time (along hydroperiod and of the three hydroperiods considered), as pond morphometry and water quality changed along with the drying out process. The reported ponds' areas correspond to the maximum pond surface calculated with 'Google Maps Area Calculator Tool'.

Pond Name	ID	Loc	Chapter	Lat (DMS)	Long (DMS)	Depth (cm)	Area (m²)	EC (mS·cm-1)	*ECELS* index	*QAELS* index
Bassa del fartet	BF	AE	1 & 2	42°13′39″ N	03°06′18″ E	60.0	2413.0	10.07	85	0.25
La Rubina	RUB	AE	1	42°15′16″ N	03°08′18″ E	39.0	162.6	12.48	75	0.46
Camping out	CO	AE	1 & 2	42°14′00″ N	03°07′07″ E	47.0	1529.0	31.06	78	0.55
Camping nord	CN	AE	1 & 2	42°14′11″ N	03°07′14″ E	110.0	6221.0	40.97	48	0.56
Camping sud	CS	AE	1 & 2	42°14′08″ N	03°07′16″ E	150.0	9970.0	43.03	43	0.55
Túries	TU	AE	1 & 2	42°14′09″ N	03°06′46″ E	29.0	68150.0	45.85	98	0.53
Connectada	CON	AE	1	42°13′54″ N	03°06′54″ E	23.0	13010.0	53.69	100	0.49
Bassa de la llúdriga	LLU	AE	1 & 2	42°15′38″ N	03°08′38″ E	38.0	3579.0	59.27	91	0.53
Bassa de l'anguila	AN	AE	1 & 2	42°15′32″ N	03°08′43″ E	44.0	5410.0	66.89	96	0.54
Fangassos	FAN	AE	1 & 2	42°15′51″ N	03°08′23″ E	16.0	445.9	69.10	83	0.25
Fra Ramon	FR	MIMBT	1 & 2	42°01′44″ N	03°11′28″ E	25.0	10870.0	40.62	78	0.51
Bassa del Pi	BPI	MIMBT	1 & 2	42°01′42″ N	03°11′18″ E	74.0	147.9	54.53	70	0.45
Bassa nova	BN	MIMBT	1	42°01′51″ N	03°11′33″ E	61.0	1044.0	26.37	85	0.54
Rajoleria	RAJ	ALB	3	42°23′48″ N	02°58′47″ E	61.2	2459.8	0.16	88	0.79
Cardonera	CARD	ALB	3	42°23′52″ N	02°58′44″ E	58.0	4977.5	0.12	96	0.81
Prats del Roser	ROS	ALB	3	42°23′44″ N	02°58′52″ E	43.4	1626.0	0.22	85	0.62

3.2 Sampling & Analysis

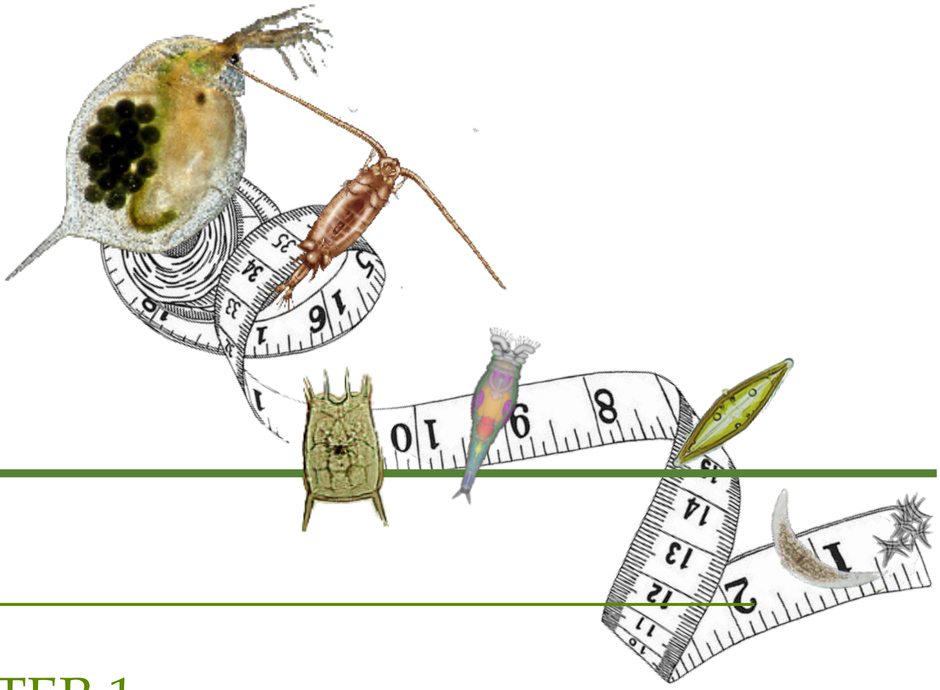
The 13 brackish ponds were sampled at the end of spring (from May to early June 2016), during the growing season (Collos et al., 2005), and when fish activity is higher (Brucet et al., 2012; Gelós et al., 2010), whereas the three temporary ponds were sampled monthly during three consecutive hydroperiods, with different length for each pond. The first hydroperiod lasted from December 2016 to May 2017, the second hydroperiod from May to June 2018, and the third from November 2018 to March 2019.

Physicochemical variables such as temperature (°C), conductivity (µS·cm^{-1}) and dissolved oxygen (%) were measured using a multiparameter probe (Hanna Instruments, Woonsocket, RI, USA). Total area (m^2) of each pond was estimated by using the "Google Maps Area Calculator Tool" (Daft Logic, 2016), whereas mean pond depth (cm) was calculated from *in-situ* repeated measures. Water transparency was estimated as Secchi depth (cm) out of maximum water column depth (cm) as it has been previously used in shallow waterbodies (Brucet et al., 2017). Water samples were analysed for total nitrogen (μmol·L^{-1}), total phosphorous (μmol·L^{-1}) and nitrates (μmol·L^{-1}), and measured according to (Sen Gupta & Koroleff, 1973), adapted by Seal Analytical to an integrated system of a CFA (Continuous Flow Analysis) digester. Chlorophyll-*a* (μg·L^{-1}) was measured using spectrophotometry after 90% acetone extraction following Parsons & Strickland (1963). Water samples for planktonic organisms were taken through the water column, collecting at least 10L from three different points and subsequently mixed in a bucket to overcome the expected patchy distribution of plankton. To obtain phytoplankton samples, 125 mL of unfiltered water were stored in 4% acid Lugol's solution, and for the zooplankton sample at least 5 L were filtered through a 50 μm mesh-size net and preserved in 4% acid Lugol's solution. Macroinvertebrate samples were obtained following standard protocols (Boix et al., 2005) and preserved in ethanol 70% (Photo S2, Supporting Information). In this protocol, macroinvertebrates are collected through a 20 cm of diameter dip-net (mesh size 250 μm). At each pond the dip-net was submerged into the water and 'pushed' rapidly for about 1 mt of length, for a total of 20 sweepings along transects to be sure to cover the different habitats of the littoral zone of the pond.

For permanent ponds different methods of fish sampling were selected to cover efficiently the different microhabitats. Shallow littoral areas were sampled using plastic minnow traps (PMT) and fyke nets (FN), a common and widely used method in coastal lagoons (Blanco et al., 2003; Franco et al., 2012; Prado et al., 2017). Multi-mesh gillnets (GN) were set on central and deepest areas of ponds that were at least 1.5 m deep since it is the height of the gillnet. PMT were made with 2 L soda plastic bottles as described in Fouilland (1996) and Clavero et al., (2006) (see Photo S3, Supporting Information). The upper piece of each bottle was cut and inverted, acting as a funnel (main characteristics: 21.5 mm of funnel diameter, 72 cm^2 of interception area, 22.5 cm of length and 9.6 cm of height). FN consisted of a semi-circular entrance ring followed by three smaller circular rings surrounded by a net (3.5 mm mesh) and had two consecutive funnels (120 mm of funnel diameter, 1050 cm^2 of interception area, 98 cm of length, 30 cm of height and 95 cm of wing length) (see Photo S4, Supporting Information). GN followed the European standard (European Committee for Standardization, 2005) composed of 12 mesh-size panels of length 2.5 m each ranging between 5 mm to 55 mm (length 30 m, height 1.5 m, knot to knot dimensions following a geometric series: 5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43, and 55 mm). PMT and FN were set during 24 h. GN were set for approximately 12 h overnight to include both evening and morning phases of high fish activity. The total number of fish traps set in each of the studied ponds varied according to their area and depth.

Fish community in the studied ponds was mainly composed by *Aphanius iberus* and the invasive fish species *Gambusia holbrooki* (Sgarzi et al., 2019). Whereas the former is, overall, more abundant in ponds of higher salinity, the latter is more abundant in oligohaline ponds (Sgarzi et al., 2019). The rest of the community is composed by the *Atherina boyeri* (Risso, 1810), *Pomatoschistus* sp. (Gill, 1863), *Mugil cephalus* (Linnaeus, 1758), the marine fish *Solea solea* (Queusel, 1806), *Anguila anguila* (Linnaeus, 1758) and the invasive *Lepomis gibbosus* (Linnaeus, 1758), although the last three species are very scarce (see (Sgarzi et al., 2019) for more details on the relative abundance of the fish species).

Fish were identified, counted, and measured *in-situ* (see Photo S5, Supporting Information), whereas macroinvertebrates, zooplankton and phytoplankton samples were analysed *a posteriori* in the university laboratory, using a stereoscope for cladoceran and copepods, and an inverted microscope for rotifers and phytoplankton (following the Utermöhl method) (see Table S5 and Table S6, Supporting Information). Phytoplankton was counted and measured following the protocol for phytoplankton identification described in the EU project 'WISER' (Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery) (Mischke et al., 2012), where, after 24h of sedimentation in the chambers, horizontal transects were counted until reaching at least 100 individuals of the most abundant taxa. From each species identified, at least 10 individuals were measured. Biovolumes for both phytoplankton and rotifers were calculated through the approximation of the body shape to geometric models (Mischke et al., 2012; Ruttner-Kolisko, 1977), and then converted into dry weight (Yvon-Durocher et al., 2011). Regarding zooplankton samples, at least 100 individuals of the most abundant taxa were counted, and the first 25 of each taxon were measured. Biomass (µg dry weight) of copepods and cladoceran was estimated by using equations of the allometric relationship between body length (in µm) and biomass (µg of dry weight) (Dumont et al., 1975; Malley et al., 1989). As there was not the necessity to dilute macroinvertebrates samples due to their low abundance (an average of 42 organisms per sample), all the individuals were counted and measured. The protocol of (Moretti et al., 2017) was employed to calculate their total length, whereas biomass (mg dry weight), was estimated following the body length-mass relationship formulas provided for the different taxonomic groups (Benke et al., 1999; Meyer, 1989; Smock, 1980; Traina & von Ende, 1992).



4. CHAPTER 1

Plankton Taxonomic and Size Diversity of Mediterranean Brackish Ponds in Spring: Influence of Abiotic and Biotic Factors

Serena Sgarzi, Anna Badosa, Àngels Leiva-Presa, Lluís Benejam, Rocío López-Flores and Sandra Brucet

4.1 ABSTRACT

In this study, performed in Mediterranean brackish ponds during spring season, we assessed the effects of biotic interactions and abiotic factors on the size and taxonomic structure of the phytoplankton and zooplankton. We used a taxonomic and a size diversity index as a descriptor of the community structure. We predicted that the size diversity of each trophic level would be mainly related to biotic interactions, such as size-based fish predation (in the case of zooplankton) and food resource availability (in the case of phytoplankton), whereas taxonomic diversity would be more affected by abiotic variables (e.g., conductivity, pond morphology). Our results showed a negative relationship between phytoplankton size diversity and food resource availability leading to low size diversities under food scarcity due to dominance of small species. Conductivity also negatively affected the phytoplankton size diversity, although slightly. Regarding zooplankton size diversity, none of predictors tested seemed to influence this index. Similar fish size diversities among ponds may prevent a significant effect of fish predation on size diversity of zooplankton. As expected, taxonomic diversity of phytoplankton and zooplankton was related to abiotic variables (specifically pond morphometry) rather than biotic interactions, which are usually body size dependent, especially in these species-poor brackish environments.

Keywords: trophic interactions; phytoplankton; zooplankton; fish; pond morphometry; food resource availability

4.2 INTRODUCTION

Mediterranean ponds are ecologically very important ecosystems that support relevant hydrological, chemical and biological processes and are biodiversity hotspots in terms of both species composition and biological traits (Céréghino et al., 2014). They are also very vulnerable habitats, because they are threatened by several anthropogenic pressures (Céréghino et al., 2008; Oertli et al., 2005). Nevertheless, these habitats have received less scientific attention than other water bodies such as lakes or rivers (Biggs et al., 2017; Boix et al., 2012).

Most ecological studies of Mediterranean ponds have been focused on their taxonomic diversity (Oertli et al., 2005), the dynamics of a certain species and communities (Anton-Pardo & Armengol, 2012; Boix et al., 2008; Brucet et al., 2012; Ruhí et al., 2009) and its relationship with nutrients dynamics and hydrological patterns (Àvila et al., 2018; Badosa et al., 2006; Brucet, Boix, et al., 2005; Gascón et al., 2005), as well as with anthropogenic pressures (Gascón et al., 2012). However, there are not many studies considering size-based interactions among adjacent trophic levels (predators and preys) of the food web (Brucet et al., 2017; Emmerson & Raffaelli, 2004) despite such interactions play a key role in the trophic structure and functioning of aquatic ecosystems (Brown et al., 2004; Brucet et al., 2017; Emmerson & Raffaelli, 2004). It is worth to mention that size-based interactions are particularly relevant in the species-poor communities like those of Mediterranean brackish ponds where trophic interactions are mainly body-size dependent (Badosa et al., 2007; Quintana et al., 2006). A way to analyze the size structure of a community is through the size diversity index, which is analogous to Shannon diversity index but adapted for continuous variables, such as body size (Brucet et al., 2006; Quintana et al., 2008). In Mediterranean brackish ponds, it has been shown that size diversity provides complementary information about the community structure to classical taxonomical approaches. While taxonomic diversity of zooplankton and macroinvertebrates communities was more sensitive to abiotic factors such as nutrient availability, size diversity was mainly related to biotic interactions (e.g., predation or inter-and/or intraspecific competition) (Badosa et al., 2007; Gascón, Boix, & Sala, 2009). However, abiotic factors can also affect the size structure of a community. For example, in other European brackish ecosystems, an increase in conductivity has been related to a decrease in zooplankton mean size and size diversity due to the replacement of large cladoceran species by copepods, small cladoceran species and rotifers, which are overall more salt-tolerant (Brucet et al., 2009; Jensen et al., 2010; Jeppesen et al., 1994).

Size structure within a trophic group of the planktonic food web can be determined by the size structure of the adjacent trophic levels since both predation and food selection are size-dependent (Brucet et al., 2017). However, each trophic group may be affected in different ways by top-down (e.g., size-based predation) or bottom-up (e.g., size diversity of resources) controls. Several studies in aquatic ecosystems have shown that fish predation is the most important driver of zooplankton size structure, as an increase in fish density is related to a decrease in density, mean body size and size diversity of zooplankton (Brucet et al., 2010; Finlay et al., 2007; Quintana et al., 2014). However, the effects of size-structured predation (i.e., predation by individuals of different sizes) on the individual size structure of prey in natural food webs are less well understood, although this information brings insight into the strength of the interactions between adjacent trophic levels and into the biomass transfer through the food web (Brucet et al., 2017; Ersoy et al., 2017; García-Comas et al., 2016). A recent study (García-Comas et al., 2016), found a negative relationship between predator (mesozooplankton) and prey (nanomicroplankton) size diversities which was explained due to the enhanced strength of top-down control at increasing predator size diversity. Nevertheless, the opposite situation has also been found - positive relationship between the size diversity of predators (fish) and prey (zooplankton) - suggesting that a higher diversity of sizes in consumers may promote diversification of resources by size (Brucet et al., 2017). On the other hand, size diversity of a trophic group may also be determined by bottom-up control since low resource availability often results in higher size diversity of consumers, as has been found in zooplankton and fish communities (Brucet et al., 2006; Quintana et al., 2014). Thus, when resources are scarce, the competitive pressure for resources between small and large individuals can be reduced by size-based selection of food, where large and small predators tent to prefer large and small prey respectively, allowing the coexistence of a wide range of organism sizes (Brucet et al., 2006, 2008; Muñoz & Ojeda, 1998; Quintana et al., 2014). Concerning phytoplankton, recent studies have shown that its size diversity is mainly determined by changes in resource availability, rather than by predation, as due to the prevailing influence of abiotic factors in their nutrient uptake (Brucet et al., 2017; Ersoy et al., 2017; Marañón et al., 2015; Quintana et al., 2014).

Whereas size diversity is usually more sensitive to biotic interactions, taxonomic diversity had been found to be more related to abiotic factors, such as nutrient concentration (Badosa et al., 2007; Gascón, Boix, & Sala, 2009; Mouillot et al., 2005), conductivity (Gascón, Boix, & Sala, 2009; Mouillot et al., 2005) and water body size (Oertli et al., 2002). In shallow ecosystems, zooplankton and benthic macroinvertebrates taxonomic diversity was found to be negatively related to increasing concentrations of phosphorus (Badosa et al., 2007; Jeppesen et al., 2000) and total organic carbon (Gascón, Boix, & Sala, 2009) respectively, whereas nitrogen and phosphorous were found to decrease phytoplankton taxonomic diversity (Romo & Villena, 2005). Regarding conductivity effects, in Mediterranean brackish communities a decrease in taxonomic diversity of zooplankton (Gascón, Boix, & Sala, 2009, Mouillot et al., 2005), macroinvertebrates (Brucet et al., 2012), and phytoplankton (López-Flores et al., 2014) was observed at highest conductivities. In these communities, conductivity plays an important role for shaping species composition and food web interactions (Alcaraz, Bisazza, et al., 2008; Brucet et al., 2010; Jensen et al., 2010; Jeppesen et al., 1994, 2007). Increased conductivity is usually related to reduced richness due to low osmoregulatory ability of several species (Anton-Pardo & Armengol, 2012, Brucet et al., 2009, Cognetti & Maltagliati, 2000, Jeppesen et al., 1994, Moss, 1994). Significant positive regression between area of the water body and the number of species had been found for ponds gastropods (Brönmark, 1985), for macroinvertebrates in streams and ponds (Brönmark et al., 1984, Oertli et al., 2002), and for phytoplankton and zooplankton in lakes (Dodson, 1991, 1992, Fryer, 1985; Jeppesen et al., 2000,). In a study on Danish shallow lakes, lake depth was also positively related to phytoplankton species richness (Jeppesen et al., 2000).

Although both taxonomic and size-based approaches can be used to complement each other, studies dealing with their combination when determining plankton community-structuring factors are still scarce (Badosa et al., 2007). The combination of both approaches becomes especially relevant in species-poor communities, like those in Mediterranean brackish ecosystems, where harsh environmental conditions make size-based interactions more relevant (Gascón, Boix, & Sala, 2009). In this study, performed in Mediterranean brackish ponds, we aimed to determine the effects of biotic interactions (i.e., predation, food resource availability) and abiotic factors (environmental variables) on the phyto- and zooplankton taxonomic and size diversity indexes, and explore if they are affected by the same factors. To analyze the effects of biotic interactions we searched for relationships among diversity metrics of adjacent trophic levels (phytoplankton, zooplankton, and fish) as previous studies did (Brucet et al., 2017; García-Comas et al., 2016). The study was carried out during the spring season since the plankton peak is broader (Collos et al., 2005), and fish activity is higher (Brucet et al., 2012; Gelós et al., 2010). We expected that taxonomic diversity for both phyto- and zooplankton would be more related to abiotic factors (e.g., conductivity, depth, area, etc.) (Badosa et al., 2007; Gascón, Boix, & Sala, 2009) whereas size diversity would be mainly related to biotic interactions (top-down and bottom-up processes). Regarding zooplankton size diversity, we expected that it would mainly be driven by top-down effects (i.e., fish predation) due to size-based predation pressure by fish. At increasing predator size diversity, top-down control would be enhanced (García-Comas et al., 2016; Ye et al., 2013) and, therefore, we predicted a negative relationship between the zooplankton and fish size diversity. In the case of phytoplankton, we expected that the size diversity would be more affected by bottom-up effects (i.e., changes in resource availability) rather than by top-down effects (i.e., zooplankton predation), as it has been observed in previous studies (Brucet et al., 2017; Ersoy et al., 2017; López-Flores, Boix, et al., 2006), and therefore we predicted a negative relationship between the size diversity of phytoplankton and its resource, due to competitive interactions for resources in less productive systems (Brucet et al., 2017).

4.3 MATERIALS & METHODS

4.3.1 Sampling & Analysis

A total of 13 permanent ponds (see Table S1 in Supporting Information) were sampled once during the spring season (May to early June 2016). We chose to sample the ponds in spring since the plankton peak is broader (Collos et al., 2005), and fish activity is higher (Brucet et al., 2012; Gelós et al., 2010). Mean water column depth (cm) was calculated from repeated measures of water column depth obtained in situ using a two-meter rule. Total area (m^2) was estimated in each pond by using 'Google Maps Area Calculator Tool' (Daft Logic, 2016). Physicochemical variables such as temperature (°C), pH, conductivity ($mS \cdot cm^{-1}$), total dissolved solids ($mg \cdot L^{-1}$) and dissolved oxygen ($mg \cdot L^{-1}$) were measured in situ using a multiparameter probe (Hanna Instruments, Woonsocket, RI, USA). Water transparency was estimated as Secchi depth (cm) out of maximum water column depth (cm) as it has been previously used in shallow waterbodies (Brucet et al., 2017). Water samples were analyzed for total phosphorus ($mg \cdot L^{-1}$), soluble reactive phosphates ($mg \cdot L^{-1}$), total nitrogen ($mg \cdot L^{-1}$) and nitrates ($mg \cdot L^{-1}$), according to Koroleff 1973 (Sen Gupta & Koroleff, 1973), adapted by Seal Analytical to an integrated system of CFA digester. Chlorophyll-*a* was measured using spectrophotometry after 90% acetone extraction following Parsons and Strickland, 1963 (Parsons & Strickland, 1963).

In each pond, water samples for planktonic organisms were taken through the water column by means of a 6 L container. Several subsamples were obtained in each pond from different sites and subsequently mixed to overcome the expected patchy distribution of plankton. To obtain a phytoplankton sample, 250 mL of unfiltered water were stored in 4% acid Lugol’s solution, and for the zooplankton sample 5 L were filtered through a 50 μm mesh-size net and preserved in 4% acid Lugol’s solution. Zooplankton individuals (including rotifers, copepods and cladocerans) were counted, identified to species level (whenever possible), and measured (total length in μm) using a stereoscope and an inverted microscope (Utermöhl method). For the individual counting and identification, we analyzed the whole sample, whereas for the measuring, we measured the first 100 individuals (when possible) assuming all individuals were equitably distributed in the observed sample. Phytoplankton was counted and identified to specie level under inverted microscope using Utermöhl chambers following the protocol for phytoplankton identification described in the EU project ‘WISER’ (Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery) (Mischke et al., 2012) When identification at species level was not possible, the different species of the same genus were numbered differently in order to respect the diversification. For both zooplankton and phytoplankton, at least 100 individuals of the most abundant taxa were counted (see Table S5, Supporting Information). For phytoplankton, biovolume was estimated through the calculation of similar geometric models (Sun & Liu, 2003).

4.3.2 Fish sampling

Different methods of fish sampling were selected to cover efficiently the different microhabitats of ponds. Shallow littoral areas were sampled using plastic minnow traps (PMT) and fyke nets (FN). Multi-mesh gillnets (GN) were set on central and deepest areas of ponds that were at least 1.5 m deep since it is the height of the gillnet. PMT were made with 2 L soda plastic bottles as described in Fouilland & Fossati, 1996 (Fouilland, 1996) and Clavero et al., 2006 (Clavero et al., 2006). The upper piece of each bottle was cut and inverted, acting as a funnel (main characteristics: 21.5 mm of funnel diameter, 72 cm^2 of interception area, 22.5 cm of length and 9.6 cm of height). FN consisted of a semicircular entrance ring followed by three smaller circular rings surrounded by a net (3.5 mm mesh) and had two consecutive funnels (120 mm of funnel diameter, 1050 cm^2 of interception area, 98 cm of length, 30 cm of height and 95 cm of wing length). GN followed the European standard (CEN, 2005) composed of 12 mesh-size panels of length 2.5 m each ranging between 5 mm to 55 mm (length 30 m, height 1.5 m, knot to knot dimensions following a geometric series: 5, 6.25, 8, 10, 12.5, 15.5, 19.5, 24, 29, 35, 43, and 55 mm). PMT and FN were set during 24 h. GN were set for approximately 12 h overnight to include both evening and morning phases of high fish activity (CEN, 2005). The total number of fish traps set in each of the studied ponds varied according to their area and depth (Table S1). Captured fish were identified (see Table S5, Supporting Information), measured for total length (mm), and released. In each studied pond, captures per effort unit (CPUE) were calculated for each fish species and each sampling method by dividing the captures for the number of traps, fyke nets or gillnets respectively.

4.3.3 Calculation of Diversity Metrics

For each phytoplankton, zooplankton, and fish samples we obtained two diversity measures: the Shannon diversity index (taxonomic, at species level), and the size diversity index (non-taxonomic). The taxonomic index (H') was calculated using the numerical abundance of each identified taxon as following (Pielou, 1969):

$$H' = -\sum_{i=1}^{S} pi \ln pi \tag{1}$$

where pi is the proportion of individuals belonging to the ith taxon, and S is the total number of identified taxa. Shannon diversity index (H') was calculated with the 'vegan' R-package (Oksanen et al., 2013).

The size diversity index (μ) was calculated using individual size measurements as proposed by Quintana et al., 2008 (Quintana et al., 2008). This index is computed based on the Shannon diversity expression adapted for a continuous variable, such as body size. This measure takes the form of an integral involving the probability density function of the size of the individuals described by the equation

$$\mu = -\int_{0}^{\infty} \rho_x (x) log_2 \, \rho_x(x) dx \tag{2}$$

where $\rho_x(x)$ is the probability density function of size x. The nonparametric Kernel estimation was used as a probability density function, which is applicable to any type of size distribution. Before computing size diversity, data were automatically standardized by division of each size value by the geometric mean of the size distribution. The size diversity index is the continuous analogue of the taxonomic Shannon diversity index, and it produces values in a similar range to those of the Shannon index. For each trophic level, we randomly measured at least 100 individuals in each sample that represents a size diversity error estimation lower than 10%. Size diversity index was computed following an R code provided by Quintana et al., (2008).

4.3.4 Data analysis

We used general linear models (GLMs) to test the effects of biotic interactions (e.g., top-down and bottom-up effects) and abiotic factors on the phyto- and zooplankton taxonomic and size diversity indexes. Thus, at each trophic level (phytoplankton and zooplankton) two GLM models were carried out considering taxonomic and size diversity indexes as response variables respectively. Since the small sample size (N = 13) limited the number of independent variables to be included in each GLM model, we used the smallest number of predictors which allowed us to test for our hypothesis: two variables reflecting biotic interactions (top-down and bottom-up effects, respectively) and three abiotic environmental variables.

As predictor variables reflecting biotic interactions, we used the two diversity indexes of the adjacent trophic levels, taxonomic or size-based depending on the response variable (i.e., size-based metrics when size diversity was the response variable and vice versa). Therefore, predictor variables used to test top-down effects (i.e., predation from upper trophic level) in phytoplankton GLMs were the zooplankton diversity indexes, and in zooplankton GLMs were the fish diversity indexes. For what concern the fish community, we only considered those planktivorous fish species since we are testing predation effects on zooplankton. We only used captures from PMT since they are more effective than FN in capturing small juveniles (Clavero et al., 2006). To test for bottom-up effects (i.e., food resource availability), predictor variables used in zooplankton GLMs were the phytoplankton diversity metrics whereas in phytoplankton GLMs we used the "phytoplankton biomass: total nitrogen" ratio. Similar ratios (e.g., Chlorophyll-*a*: TP, Chlorophyll-*a*: TN) have been previously used in studies dealing with phytoplankton yield, as a measure of the phytoplankton resources limitation (ratio values are high under resource limitation) (Brucet et al., 2009, 2017; Jeppesen et al., 2003).

As predictor abiotic variables for both phyto- and zooplankton GLMs, we considered conductivity, pond area, and depth because of the absence of correlation among them and with the diversity metrics (Pearson's $r < |0.31|$ and p-value > 0.30 for all cases. See supplementary table S2 for correlations details). These three abiotic variables have already been shown to play an important role in structuring Mediterranean pond communities (Brucet et al., 2008, 2017; Ersoy et al., 2017). We dismissed using other abiotic variables that, although not correlated, did not show enough variation among ponds (e.g., temperature, pH). The rest of the measured environmental variables were significantly correlated among them (Pearson's $r > |0.56|$ and p-value < 0.05 for all cases).

We looked for the most parsimonious of the full models by performing an automatic backward selection of one predictor variable at a time by minimizing the Akaike information criteria (AIC). The most parsimonious model was the combination of variables having the strongest impact on outcomes. To compare the relative strength of the significant predictors, we calculated their standardized (beta) coefficients, and adjusted R^2 was used as a measure of the variability explained by the model. Variables were log-transformed when necessary to improve linearity and reduce heteroscedasticity, and residual plots were inspected to detect violations of regression assumptions. The variance inflation factor (VIF) was used to check collinearity, and Cook's distances were analyzed to check for outliers and influential cases. We are aware that a low number of observations (N = 13) prevent making reliable predictions when performing multiple regression models taking the risk to incur into Type I error. Nevertheless, to make an estimation of the statistical power of the GLM models obtained, we computed the statistical power analysis of each model using the G*Power software (Erdfelder et al., 2009)

All data have been analyzed using R version 3.4.2 (R core Team, 2017, Boston, MA, USA)~~.~~ All plots have been created with 'ggplot2' R-package (Wickham, 2016).

4.4 RESULTS

Physico-chemical variables measured in the studied ponds are shown in Table 1. Since we sampled once during the spring season, water temperatures (°C) were relatively similar between ponds (with a mean value of 25.56 °C and a standard deviation of 2.97), as well as pH. Studied ponds are brackish, with conductivity values ranging from 10.7 $mS \cdot cm^{-1}$ to 69.10 $mS \cdot cm^{-1}$, and shallow, with mean depths ranging from 16 cm up to 150 cm. In addition, they differed quite a lot also in their areas, with values ranging from 147.90 m^2 to 68,150 m^2. The dissolved oxygen content in water measured during daytime showed a situation very far from anoxia, with values from 41.10 $mg \cdot L^{-1}$ to 262.50 $mg \cdot L^{-1}$. Chlorophyll-*a* values ranged from a minimum value of 0.31 $\mu g \cdot L^{-1}$ to a maximum value of 43.41 $\mu g \cdot L^{-1}$, and the mean water transparency, measured as Secchi depth: maximum depth, was 0.89 cm. With regard to nutrient concentrations, inorganic forms of nitrogen and phosphorus (nitrate and soluble reactive phosphate) were always lower than total nitrogen and phosphorus (about 400 and 3 times lower respectively), which included organic and inorganic forms (Table 2). Nitrates were especially lower in the studied ponds with an average concentration of 0.21 $\mu mol \cdot L^{-1}$. Values of the ratio "phytoplankton biomass: total nitrogen", as a measure of resource limitation for the phytoplankton, ranged from 0.09 (high resource availability) to 1.58 (low resource availability).

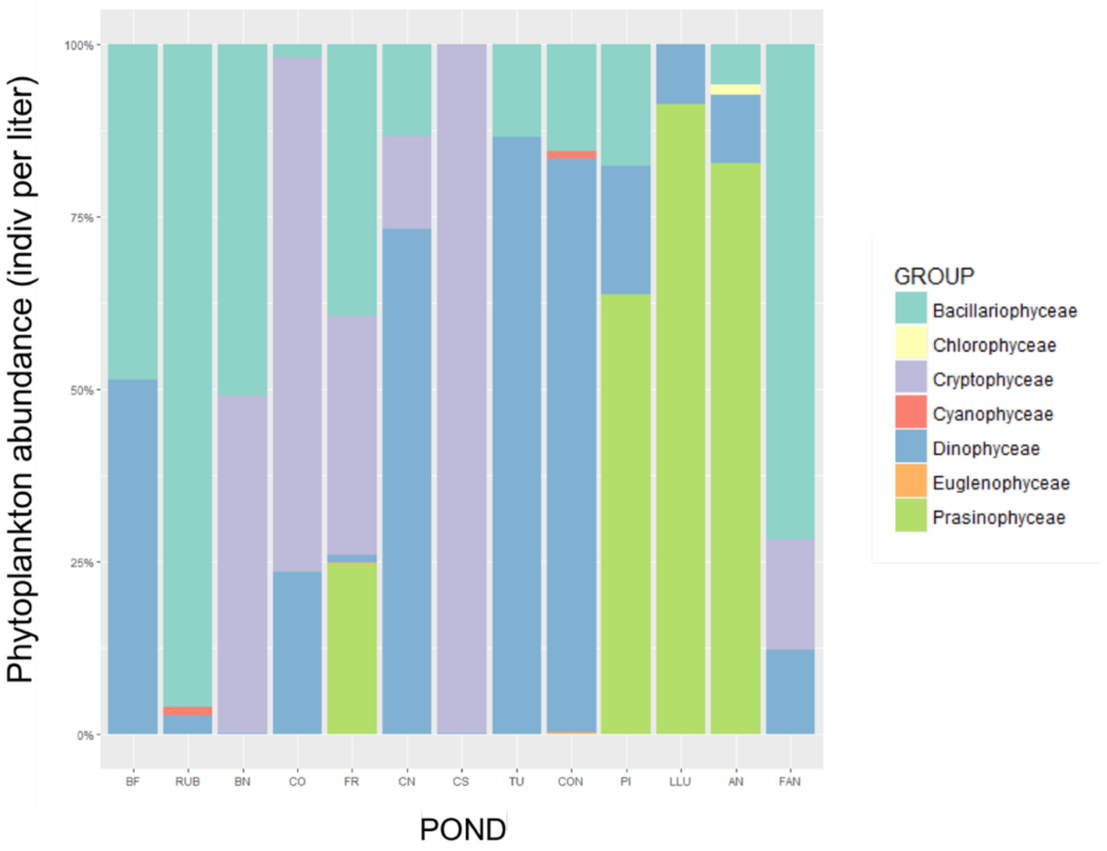
With regards the phytoplankton community in the studied ponds, a total of 39 species were identified belonging to seven classes (Figure 6). Prasinophyceans (mostly marine species) dominated the community (>60%) under high conductivity (ponds PI: 'Bassa del Pi', LLU: 'Bassa de la llúdriga' and AN: 'Bassa de l'anguila'), whereas bacillariophyceans (diatoms) were more abundant in the 3 ponds that showed lower conductivities, with the specie *Navicula* sp. (Bory de Saint-Vincent, 1822), except for FAN (Fangassos) pond where diatoms were probably composed by marine species. In the rest of the ponds, phytoplankton was dominated by cryptophyceans and dinophyceans (dinoflagellates). Phytoplankton individual length ranged from 2.55 μm (cyanophiceans) to 112.81 μm (prasinophyceans).

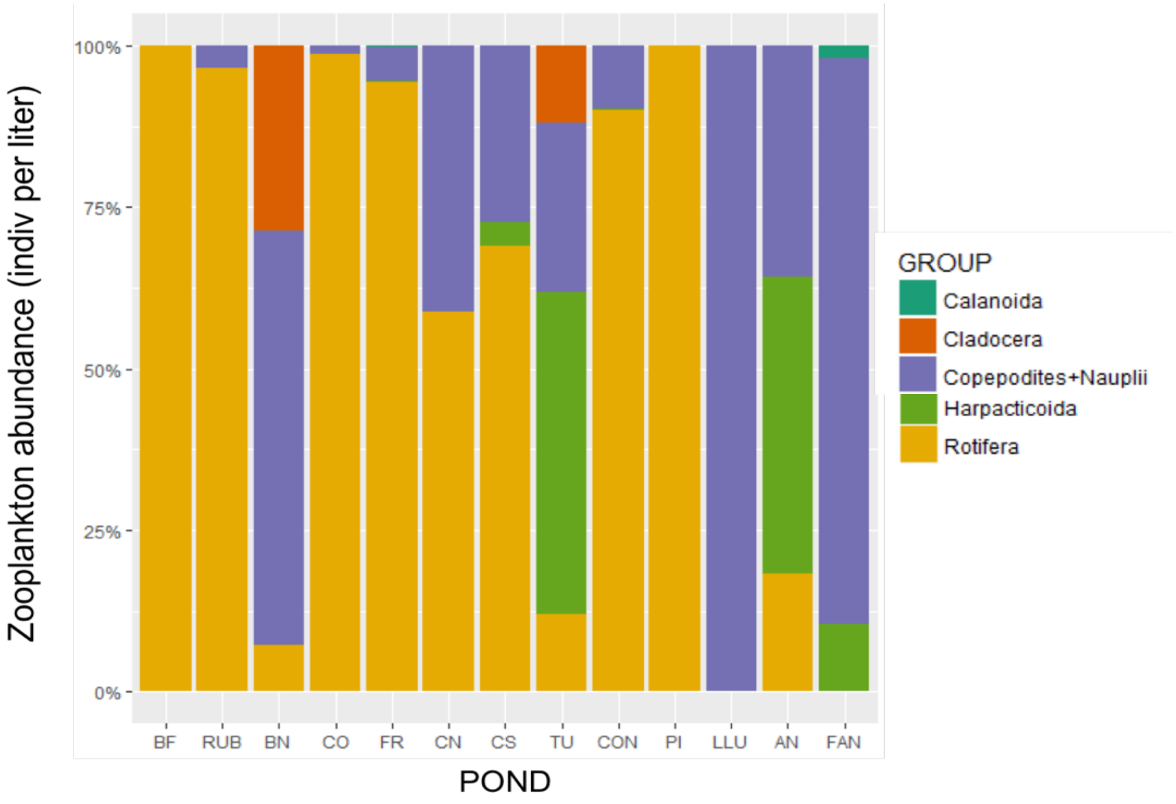
Regarding the zooplankton community, a total of 17 species were found in the studied ponds being rotifers the dominant group in 8 of the ponds (>50% of total zooplankton abundance, Figure 6). In the other five ponds, the zooplankton was dominated (>70% to 100%) by the group of copepods, where planktonic larval stages (copepodites + nauplii) were the dominant followed by adult individuals of calanoids and harpacticoids. Cladocerans were the less abundant and frequent (present only in two ponds) and did not dominate the community in any pond. Zooplankton individual length ranged from 0.39 mm (rotifers) to a maximum of 5.54 mm (cladocerans).

For what concern the fish community, we found 8 species including both continental and marine species, being the latter the less abundant. The endemic *Aphanius iberus* (Valenciennes, 1846), an omnivorous an euryhaline species, was well distributed along the conductivity gradient and was the most abundant species reaching a maximum of 957.6 CPUE in all the ponds together. It was present in 11 out of 13 ponds and dominated the community (>70%) in 8 ponds (Figure 6). The second most abundant fish was the freshwater, invasive planktivorous *Gambusia holbrooki* (Girard, 1859) that reached a maximum total abundance of 705.9 CPUE in all the ponds together. It was only present in the less salty ponds (Figure 6) except in the pond BF (Bassa Fartet) where the species never arrived. In order of abundance, the following species were the planktivorous *Atherina boyeri* (Risso, 1810), with a maximum CPUE of 102.9, the benthivorous *Pomatoschistus sp*. (Gill, 1863) with a maximum CPUE of 62.2 and *Mugil cephalus* (Linnaeus, 1758) with a CPUE of 41.8, all of them present in five ponds at most. The less abundant (≤4.8 CPUE) and frequent (<2 ponds) species were, in order, the marine fish *Solea solea* (Queusel, 1806), *Anguila anguila* (Linnaeus, 1758) and the invasive *Lepomis gibbosus* (Linnaeus, 1758). Fish individual length ranged from 6 mm (*A. iberus* juveniles) to a maximum of 60 cm (*A. anguila*).

Table 2. Mean, minimum, maximum, and standard deviations (SD) values of the physico-chemical and morphometric variables measured in the study ponds (N = 13). The "phytoplankton biomass: total nitrogen" ratio used in phytoplankton GLMs models to test the bottom-up effects (i.e., food resource availability, see text Section 2.4) is also included.

	Mean	**Minimum**	**Maximum**	**SD**
Mean water column depth (cm)	55.08	16.00	150.00	37.99
Pond area (m^2)	9457.90	147.90	68,150.00	18,162.80
Water transparency	0.89	0.64	1.00	0.12
Temperature (°C)	25.56	21.14	32.20	2.97
pH	8.47	7.39	9.97	0.72
Conductivity ($mS \cdot cm^{-1}$)	42.61	10.07	69.10	18.74
Total dissolved solids ($g \cdot L^{-1}$)	0.03	0.01	0.05	0.00
Dissolved oxygen (%)	115.04	41.10	262.50	56.66
Chlorophyll-a ($\mu g \cdot L^{-1}$)	11.92	0.31	43.41	14.54
Total nitrogen ($\mu mol \cdot L^{-1}$)	97.00	58.55	234.40	47.46
Total phosphorus ($\mu mol \cdot L^{-1}$)	3.19	0.44	7.57	2.15
Nitrate ($\mu mol \cdot L^{-1}$)	0.21	0.07	0.66	0.16
Soluble reactive phosphate ($\mu mol \cdot L^{-1}$)	1.27	0.09	6.94	2.01
Phytoplankton biomass: total nitrogen	0.35	0.09	1.58	0.42


Phytoplankton abundance (indiv per liter)
100%
75%
50%
25%
0%
BF
RUB
BN
CO
FR
CN
CS
TU
CON
PI
LLU
AN
FAN
POND
GROUP
Bacillariophyceae
Chlorophyceae
Cryptophyceae
Cyanophyceae
Dinophyceae
Euglenophyceae
Prasinophyceae


Zooplankton abundance (indiv per liter)
100%
75%
50%
25%
0%
BF
RUB
BN
CO
FR
CN
CS
TU
CON
PI
LLU
AN
FAN
POND
GROUP
Calanoida
Cladocera
Copepodites+Nauplii
Harpacticoida
Rotifera

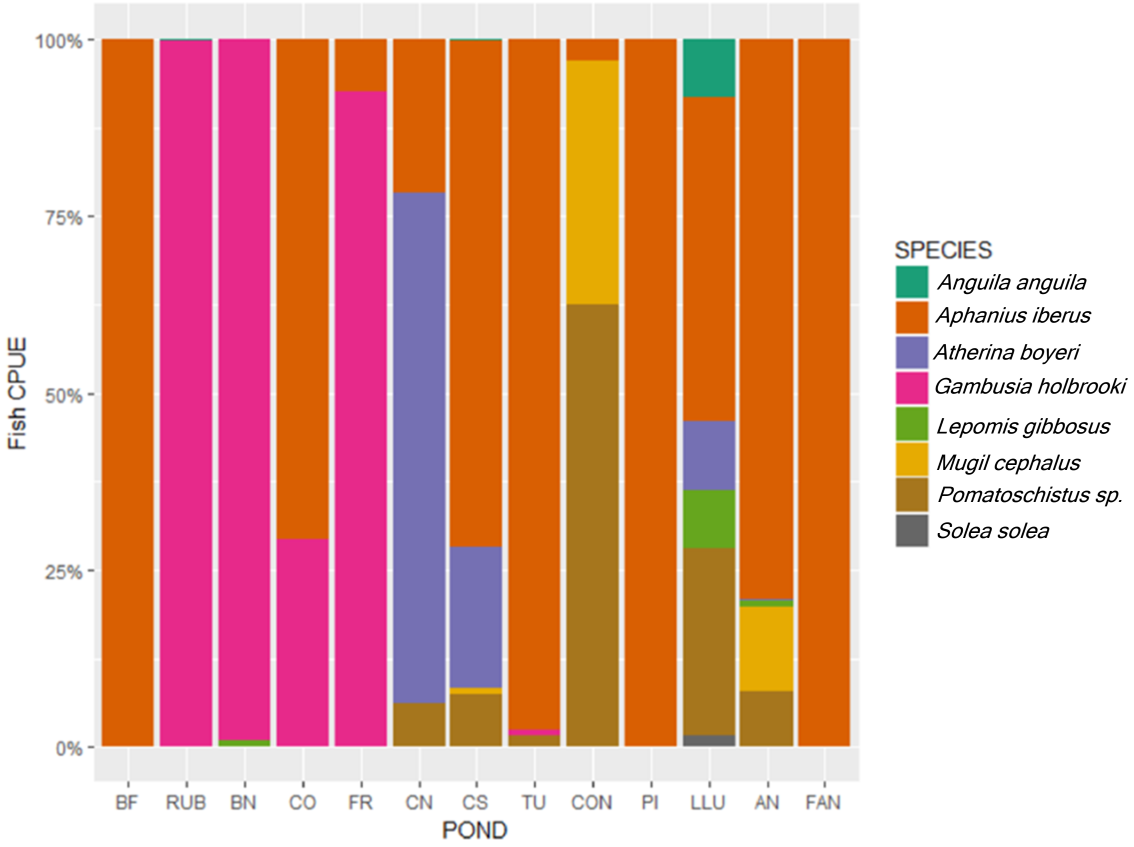


Figure 6. Relative abundance (ind·L^{-1}) of the main groups of (**upper plot**) phytoplankton and (**middle plot**) zooplankton, and (**lower plot**) fish species (CPUE) in the 13 studied ponds ordered by increasing conductivity (from left to right). Note that for the zooplankton bar graph, larval stages of copepods (copepodites + nauplii) include all groups, calanoids and harpacticoids. Abbreviations of ponds' names are explained in Table 1.

Diversity metrics calculated for the three trophic levels (phytoplankton, zooplankton, and fish) are shown in Table 3. Size diversity values ranged from a minimum of 0.31 up to a maximum of 2.95, both found in phytoplankton assemblages, while taxonomic diversity ranged from 0.00 (in those zooplankton and fish assemblages composed by one species), to a maximum of 2.14 (in phytoplankton assemblages).

Table 3. Diversity metrics of phytoplankton, zooplankton, and fish assemblages in the studied ponds (N = 13). The descriptive statistics are the mean, minimum, maximum, and standard deviation (SD). Fish diversity metrics were computed using only those planktivorous species (*A. iberus*, *G. holbrooki, A. boyeri*).

	Mean	**Minimum**	**Maximum**	**SD**
Phytoplankton				
Size diversity	1.79	0.31	2.95	0.61
Taxonomic diversity	0.94	0.02	2.14	0.53
Zooplankton				
Size diversity	1.95	0.70	2.84	0.67
Taxonomic diversity	0.38	0.00	1.13	0.34
Fish				
Size diversity	1.42	0.72	2.08	0.37
Taxonomic diversity	0.08	0.00	0.40	0.14

Variables significantly affecting size and taxonomic diversity in each of the GLM models performed are shown in Table 4. According to GLMs results (Table 4, Figures 8, 9, and see the full models in Table S2 in Supporting Information) in the phytoplankton assemblages, size diversity was slightly positively related with conductivity (marginally significant *p*-value = 0.06), and negatively related with the ratio "phytoplankton biomass: total nitrogen" (*p*-value = 0.02) (Figure 7). Beta coefficients showed values of 0.48 for conductivity and −0.62 for the ratio meaning that the latter had a stronger effect on the dependent variable. This model explained 47% of the variation in the size diversity (*p*-value = 0.04, statistical power = 0.55). Taxonomic diversity in the phytoplankton was positively related with area and negatively related with depth of the pond (Figure 8. In this second model, beta coefficients showed values of 0.46 for pond area and −0.73 for the pond depth showing a stronger effect of pond depth. This model explained 76% of the variance in the phytoplankton taxonomic diversity (*p*-value < 0.01, statistical power = 0.99). Regarding zooplankton assemblages, none of predictor variables tested were selected in the GLMs to be significant for size diversity. Instead, taxonomic diversity was positively related with the area of the pond (Figure 8). A Beta coefficient of 0.65 showed a high strength of the effect of pond area on the dependent variable. The model explained 42% of variance (*p*-value= 0.02, statistical power = 0.80.)

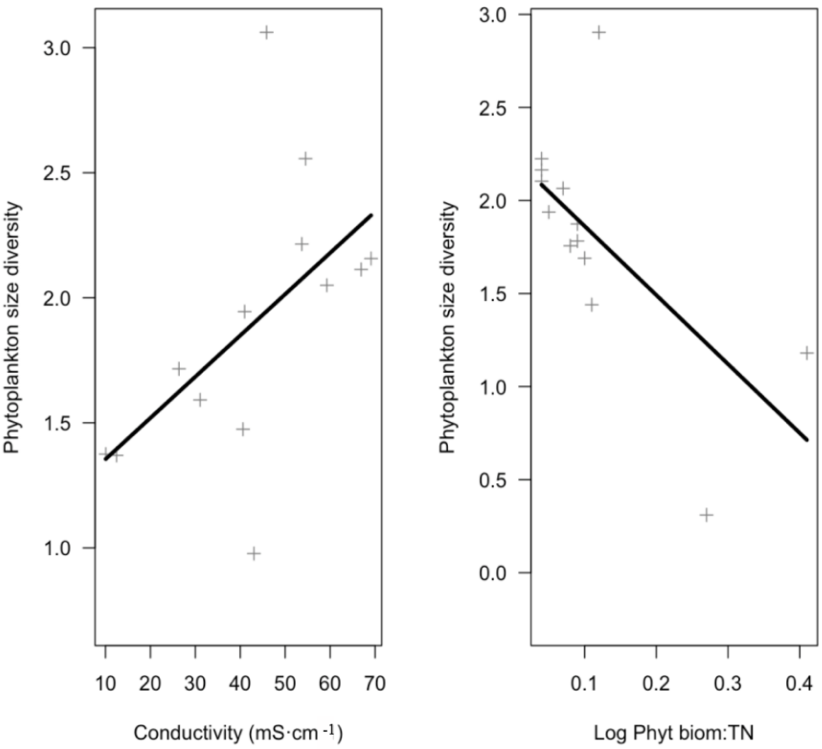


Figure 7. Partial plots obtained from GLM models showing the relationships between size diversity of phytoplankton assemblage and those predictor variables selected (see Table 4).

Table 4. Results of the GLMs (N = 13) showing the predictor variables that affect size diversity and taxonomic diversity of phytoplankton and zooplankton assemblages. Only the most parsimonious significant models are presented. For each one, intercept (estimate and standard error, S.E.), Beta coefficients (standardized), *t*-value, significance (*p*-value) and degrees of freedom (df) are shown. For each model, the global *p*-value, R^2 and the statistical power are shown. Phytoplankton biomass: TN is the ratio of "phytoplankton biomass: total nitrogen" used as a measure of food resource availability for phytoplankton.

Response Variable	Predictor	Estimate	S.E.	Beta Coefficients	*t*-Value	*p*-Value	df	Global *p*-Value	R^2	Statistical Power
Phytoplankton										
Size diversity	Conductivity	0.02	0.01	0.48	2.04	0.06	11	0.04	0.47	0.55
	Log Phytoplankton biomass:TN	−3.71	1.42	−0.62	−2.62	0.02				
Species diversity	Log Pond Area	0.32	0.11	0.46	2.96	0.01	11	<0.01	0.76	0.99
	log Mean Depth	−1.44	0.31	−0.73	−4.73	<0.01				
Zooplankton										
Species diversity	Log Pond area	0.29	0.10	0.65	2.86	0.02	11	0.02	0.42	0.8

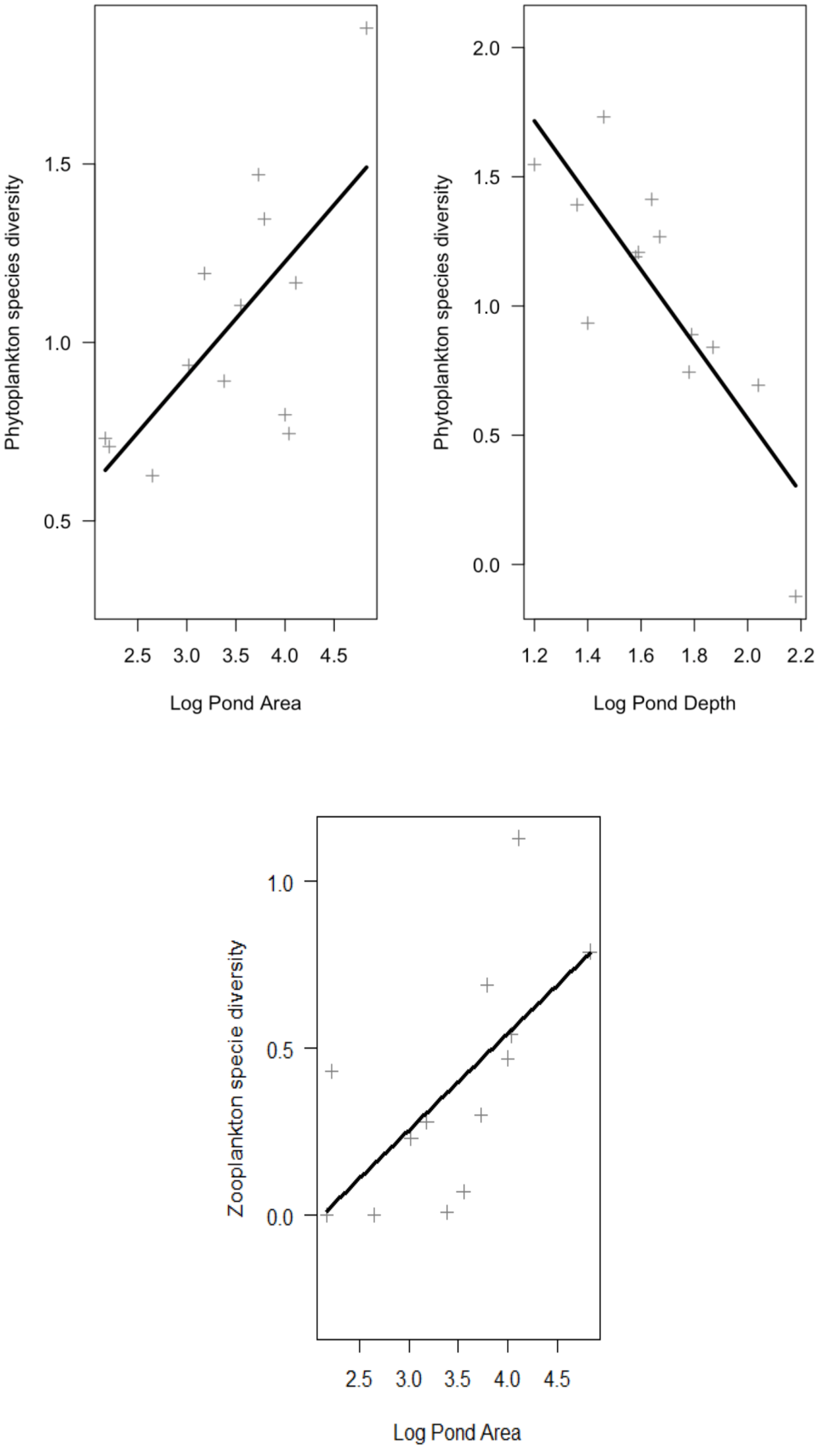


Figure 8. Partial plots obtained from GLM models showing the relationships between taxonomic diversities of phytoplankton (upper graphics) and zooplankton (lower graphic) and those predictor variables significantly related with the response variable (see Table 4).

For a graphical visualization of the results obtained, see Figure S4 in Supporting Information.

4.5 DISCUSSION

In the studied ponds, the physico-chemical characteristics of the water, as well as the composition of planktonic communities, were the expected for Mediterranean coastal brackish ponds during spring season. Thus, they show a variable conductivity (ranges within expected for brackish waters) and low concentrations of inorganic nutrients (nitrates and phosphates) due to the absence of water inputs during the sampling period. Inorganic nutrients are quickly up taken by phytoplankton as they enter into the pond, and then transformed into organic form (López-Flores et al., 2009; Quintana et al., 1998). Therefore, high concentrations of total nutrients (both nitrogen and phosphorus) are typical in these confined ecosystems, which even tend to increase in spring and summer due to evaporation phenomena (Quintana, 2002). Regarding biological communities, the phytoplankton species found were also characteristic of the brackish coastal ponds during spring season, with a high presence of marine species (dinophyceans) that can stand high salinities and entered the ponds during sporadic flooding events (e.g., sea storms) (López-Flores et al., 2014, Quintana, 2002). Zooplankton composition was also similar to the composition found in spring in previous studies in brackish ecosystems (Badosa et al., 2006; Quintana et al., 1998) with the dominance of salt tolerance rotifer species and planktonic larval stages of copepods. Regarding fish community, marine species were found in low abundance in ponds with higher salinity, due to sporadic flooding events, whereas small planktivorous fish (*A. iberus* and *G. holbrooki*) were the dominant and more abundant species in all the studied ponds. Ranges of taxonomic and size diversity values found in each trophic level were also characteristic of the spring season in Mediterranean brackish ponds (Compte et al., 2012; García-Berthou & Moreno-Amich, 1991).

Regarding the effects of abiotic and biotic factors on plankton diversity indexes, our results agreed with our hypothesis regarding taxonomic diversity since it was significantly affected by an abiotic factor (pond morphometry) for both phytoplankton and zooplankton assemblages. Thus, in the studied Mediterranean brackish ponds, our results showed that when increasing pond area both the phytoplankton and zooplankton taxonomic diversity also increased. Phytoplankton taxonomic diversity increased also when decreasing pond depth. These results are supported by the biogeographical principle that a larger area supports more species, (MacArthur & Wilson, 1967), and that area is one of the best single predictors of species number (Møller & Rørdam, 1985). Actually, one of the most commonly observed patterns in aquatic ecology, and for a wide variate of taxa (planktonic and benthonic organisms), is the positive relationship between the diversity metrics and the area of the water body (Barbour & Brown, 1974; Brönmark, 1985; Eadie et al., 1986; Fryer, 1985). According to those studies, in a bigger pond there is likely more habitat heterogeneity, favoring the coexistence of more species. In the present study, a bigger area was translated to a more diverse littoral and aquatic habitats (e.g., presence of *Phragmites australis*, *Arthrocnemum* sp., *Juncus maritimus*, *Ruppia* sp., algal mats, unvegetated open waters, etc..). In a study on biodiversity in Danish lakes (Jeppesen et al., 2000), lake depth was also related to phyto- and zooplankton richness.

Even though conductivity varied among the studied ponds during the spring sampling (from 10.07 to 69.10 $mS\cdot cm^{-1}$), probably due to different evaporation rates (different surface, wind influence) (Quintana, 2002; Quintana et al., 1998) or water inputs during previous flooding events (fresh- or seawater inputs) (Martinoy et al., 2006; Quintana, 2002), we did not find a significant effect of conductivity on phyto- and zooplankton taxonomic diversity. This may suggest that conductivity values did not differ enough among ponds to have an appreciable effect on the taxonomic diversity of plankton. Conductivity has been widely recorded as a main environmental factor shaping taxonomic structure and biodiversity in Mediterranean communities (Boix et al., 2008) having negative consequences in species composition, such as a reduction in species richness and diversity, and also affecting the food web interactions (Brucet et al., 2009, 2010).

Concerning the effects of the analyzed environmental factors on the plankton size diversity in the spring season, results obtained in the present study partially agreed with our hypothesis that size diversity (in contrast to taxonomic diversity) was more related to biotic interactions (predation and/or food resource availability). However, this was only true for phytoplankton assemblages since for zooplankton none of the abiotic nor biotic predictor variables tested significantly affected the size diversity. As we predicted, our results showed that phytoplankton size diversity was mainly affected by bottom-up effects (i.e., food resource availability) rather than top-down effects (i.e., predation), as it has been found in previous studies in lakes (Brucet et al., 2017; Ersoy et al., 2017; Quintana et al., 2014) and marine ecosystems (Garzke et al., 2015, Marañón et al., 2012, Sommer et al., 2016). In the present study, resource limitation (i.e., high values of the “phytoplankton biomass: total nitrogen” ratio) would lead to a dominance of small phytoplankton cells and, therefore, to a decrease in size diversity under low nutrient availability. Small phytoplankton is more efficient than the large one due to their low resource requirements and high cell’s surface-to-volume ratio (Guidi et al., 2009, Litchman & Klausmeier, 2008). Our results agree with previous studies in lake and marine ecosystems where changes in phytoplankton resource supplies, like total phosphorous (Brucet et al., 2017) and a resource supply index (Marañón et al., 2015), were the main drivers of phytoplankton size structure since smaller sizes are favored at low nutrient availability due to the higher surface: volume ratio or lower resource requirement. Nevertheless, there are some studies which found both positive (Fox, 2004) and negative (García-Comas et al., 2016) relationships between zooplankton and phytoplankton size diversities thus indicating a top-down control on the phytoplankton size structure. In the case of positive relationships, prey size diversity was found to increase predator size diversity, promoting diversity of consumers (Fox, 2004) whereas in the case of negative relationships, an increase in predator size diversity was found to enhance the strength of top-down control, reducing prey size diversity (García-Comas et al., 2016). It is worth mentioning that, in the present study, a slightly positive effect of conductivity on phytoplankton size diversity was also observed. The additional presence of phytoplankton marine species in high-conductivity ponds (i.e., prasinophyceans and some bacillariophyceans) likely increased the phytoplankton size diversity. The entrance of marine phytoplankton in salt marshes was also observed in López-Flores et al., 2006 (López-Flores, Garcés, et al., 2006) after a sea storm, leading to a change in the specie composition. In the present study, the pond with the less size diversity was dominated by small-sized species (e.g., cryptophyceans) suggesting that the prevalence of small-sized individuals leads to a low size diversity.

Against our predictions, zooplankton size diversity in these Mediterranean ponds during the spring season was not affected by fish size diversity (i.e., top-down effects) although previous studies have found significant relationships between predators and prey size diversity (e.g., zooplankton and planktivorous fish (Brucet et al., 2017), planktivorous and piscivorous fish (Mehner et al., 2016)). It is important to highlight that in these previous studies a wide geographic range was considered (including different ecoregions and altitudes), and predators size diversity ranges were wider than the ones found in the present study. For example, in Brucet et al., (2017) fish size diversity ranged from a minimum of −0.81 to a maximum of 2.42, whereas in our study, where geographical variation was negligible, size diversity of planktivorous fish ranged from 0.72 to 2.08. This was probably due to the similar sizes of the planktivorous fish present in the studied ponds (*A. iberus*, *G. holbrooki*, and *A. boyeri,* size ranges between 6 mm and 5.8 cm) that lead to a small range of size diversity values. This, together with the fact of not including in our analysis the presence of other predators, that although they were observed *in situ* (e.g., jellyfish *Odessia* *maeotica*, the amphipod *Gammarus aequicauda*) they could not be sampled properly, may prevent finding significant top-down effects on zooplankton size structure. We cannot ignore the fact that in the Mediterranean brackish ponds both the jellyfish and the amphipod have been found to act as planktonic top predators (Compte et al., 2010, 2011) causing changes in lower trophic levels through cascading effects (Oguz et al., 2001; Pitt et al., 2007).

In conclusion, our results suggested that in Mediterranean brackish ponds during spring season both the taxonomic diversity of phyto- and zooplankton were mainly influenced by abiotic factors (pond morphometry variables), whereas size diversity was mainly affected by biotic interactions but just in the case of phytoplankton. Nutrient limitation (i.e., bottom-up effects) rather than zooplankton predation (i.e., top-down effects) affected the phytoplankton size diversity leading to small phytoplankton size diversity when resource availability was low. Regarding zooplankton size diversity we did not find the expected top-down effects (i.e., fish predation) probably due to the similar sizes of planktivorous fish lead to similar fish size diversities among ponds. We are aware that our findings may have been limited by the low number of ponds studied, but also by the fact of considering the classical three-level food chain (phytoplankton, zooplankton and fish) neglecting the effects of omnivory and intraguild predation (Chang et al., 2014; Post & Takimoto, 2007, Quintana et al., 2014,), and the presence of the microbial loop (Persson et al., 1996; Sherr & Sherr, 1988,). It is worth mentioning that our results are limited to spring plankton communities in Mediterranean brackish ponds and we cannot rule out the effect of seasonality in our results since, in the Mediterranean region, it leads to greater intra-annual environmental variability which is also reflected in planktonic community dynamics and composition (Brucet et al., 2009; Quintana, 2002; Quintana et al., 1998).

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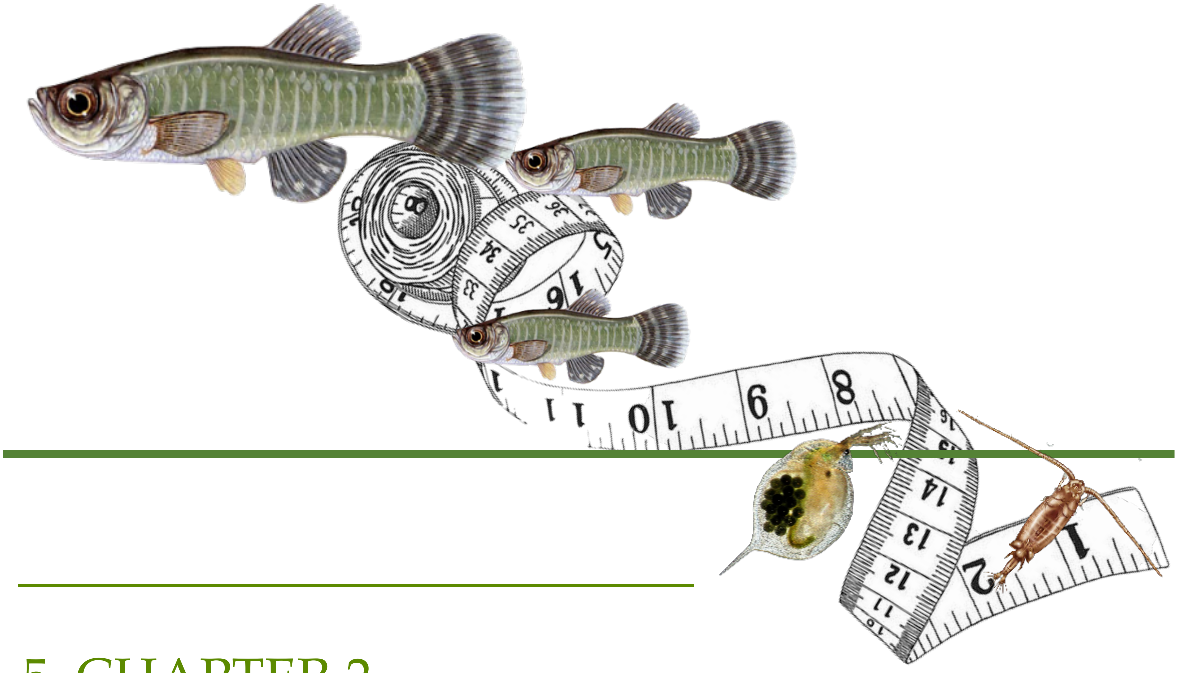

5. CHAPTER 2

Factors Influencing Abundances and Population Size Structure of the Threatened and Endemic Cyprinodont *Aphanius iberus* in Mediterranean Brackish Ponds

Serena Sgarzi, Sandra Brucet, Mireia Bartrons, Ignasi Arranz, Lluís Benejam and Anna Badosa

5.1 ABSTRACT

Aphanius iberus is an endemic cyprinodontoid fish species of Mediterranean ponds in danger of extinction. In this study, we studied some abiotic and biotic factors that can influence *A. iberus*'s size structure and density in Mediterranean brackish ponds. We sampled fish using fyke nets in 10 ponds of Empordà (Spain) during the spring season. Our results showed that a better ecological status, according to the Water Quality of Lentic and Shallow Ecosystems (*QAELS* index), pond's depth and pond's isolation (reflected by an increase in total nitrogen) were related to larger individual sizes and more size-diverse populations. Increasing the salinity is known to help the euryhaline *A. iberus* acting as a refuge from competitors. Nevertheless, our results showed that higher conductivities had a negative effect on *A. iberus*'s size structure, leading to a decrease in the mean and maximum size of the fish. Fish abundance (expressed as captures per unit of effort (CPUE)) seemed to increase with increasing the pond's depth and total nitrogen (the latter reflecting pond confinement). In conclusion, our results suggest that achieving a better pond ecological status may be important for the conservation of endangered *A. iberus*, because better size-structured populations (i.e., larger mean and maximum lengths) were found at higher water quality conditions.

Keywords: Mediterranean ponds; fish; *Aphanius iberus*; size structure; ecological status

5.2 INTRODUCTION

Aquatic ecosystems of the Iberian Peninsula are a hotspot for endemic freshwater fish fauna; still, most of the fish species are critically threatened by habitat destruction, intensive agricultural activities or the introduction of exotic species (Doadrio, 2001; Vilà & García-Berthou, 2010). The Spanish toothcarp (*Aphanius iberus*, Valenciennes, 1846) is a small cyprinodont (up to 6 cm in length) endemic from the Eastern Mediterranean lowland waters of the Iberian Peninsula (De Sostoa, 1983; Moreno-Amich, 1989; Oliva-Paterna et al., 2006) and in danger of extinction (Crivelli, 2006; Doadrio et al., 2002). As other cyprinodonts, it is characterized by fast growth, early maturity, high reproductive effort and multiple spawnings (García-Berthou & Moreno-Amich, 1993; García-Berthou & Moreno-Amich, 1992), which implies a short longevity (age up to two+). *A. iberus* is an eurytherm and euryhaline species, well-adapted to changes in environmental conditions (García-Berthou & Moreno-Amich, 1999) such as sudden alterations in temperature and salinity due to marine intrusions or freshwater floodings (Demestre et al., 1977). This cyprinodont originally inhabited a wide range of lowland waterbodies, but now, its geographical distribution is limited to brackish and hypersaline coastal waterbodies (Doadrio, 2001; García-Berthou & Moreno-Amich, 1992; Oliva-Paterna et al., 2006; Rincón et al., 2002) due to habitat degradation (e.g., intensive agriculture, water pollution and wetland desiccation) and the introduction of invasive species, which usually act in an additive manner, since habitat degradation facilitates biological invasions (Dudgeon et al., 2006; Mack et al., 2000). The high degree of isolation among the remaining populations also poses a threat to their conservation, as they show higher rates of extinction than populations in well-connected locations (Oliva-Paterna et al., 2006).

Some studies have shown that the abundance and size structure of *A. iberus* depends on the ecological status of the ponds, with larger individuals and higher densities found in ponds with a higher water quality (Casas et al., 2011). Indeed, in Italy, another species of the *Aphanius* genus (*Aphanius fasciatus*) has been proposed as an indicator of the ecological status of salt marshes (Franco et al., 2009), suggesting that those fish are sensitive to changes in the ecological status of their environments. More confined and less accessible ponds also seem to host populations of *A. iberus* more abundant and stable over time (Prado et al., 2017). Another variable that may influence the density and size structure of *A. iberus* is the pond morphometry (area and depth), because it has a strong impact on the structural complexity and niche availability, as has been found for other fish species and communities (Arranz et al., 2016; Emmrich et al., 2011; Holmgren & Appelberg, 2000). However, to our knowledge, there have been not studies in this respect.

The Eastern mosquitofish (*Gambusia holbrooki*) (Lowe et al., 2000; Pyke, 2005) is an invasive species that very often interacts with *A. iberus*, because both share similar habitats (Vargas, 1993) and compete for the same resources (Alcaraz & García-Berthou, 2007; Haiahem et al., 2017). Both fish species are zooplanktivorous, but *G. holbrooki* consumes mainly cladocerans, ostracods and copepods (Crivelli & Boy, 1987; Garcia-Berthou, 1999), and *A. iberus* prefers harpacticoid copepods, copepod nauplii and detritus (Alcaraz & García-Berthou, 2007). Nevertheless, in a study of two syntopic populations, it was observed that the diets of *A. iberus* and *G. holbrooki* are not totally overlapped, as the endemic specie consumed substantially more detritus and plant matter than *G. holbrooki*, and preyed more on benthonic animals, whereas the allochthon specie preferred surface and water column taxa (Vargas, 1993). Sometimes, *G. holbrooki* can act in an aggressive way against *A. iberus,* and this behavior seems to be inversely proportional to the salinity, as well as its ability to capture prey (Alcaraz, Bisazza, et al., 2008). Young individuals of *A. iberus* have been found to capture less prey in the presence of conspecific adults and *G. holbrooki*, suggesting both strong intraspecific and interspecific competition (Caiola & De Sostoa, 2005; Rincón et al., 2002). Currently, *A. iberus* has disappeared from fresh and oligohaline waters, and its habitat is restricted to salt marshes, coastal lagoons and river mouths (Alcaraz, Pou-Rovira, et al., 2008; Alcaraz & García-Berthou, 2007), where the invasion success of the mosquitofish is limited due to the high salinity fluctuations (Alcaraz & García-Berthou, 2007; Magellan & García-Berthou, 2016; Prado et al., 2014). Nevertheless, although *A. iberus* tolerates high salinity conditions, its metabolism may be affected when the salinity levels in the location are high (Brett & Groves, 1979; Wootton, 1989; Yildirim & Karacuha, 2008). Physiological functions such as oxygen consumption, critical swimming speed and routine activity level show a general decrease at the extreme salinity in *Aphanius dispar* (Plaut, 2000), although the spawning efficiency seems not to be significantly affected by the changes in salinity (Oltra & Todolí, 2000).

Identifying the key factors that influence the population structure of *A. iberus* is relevant to develop efficient conservation and management plans for this endangered species. The body size of *A. iberus* has been used to assess growth-related parameters, such as age (García-Berthou & Moreno-Amich, 1992), fecundity and sexual maturity (Vargas & De Sostoa, 1997), as well as ammonia excretion rates (Oliva-Paterna et al., 2007). Although, in Mediterranean brackish ponds, trophic interactions are very often body size-dependent (Badosa et al., 2007; Compte et al., 2012; Quintana et al., 2006), studies about the size structure of *A. iberus* and the factors that determine it are scarce.

The present study aims to identify the factors influencing the abundances and population size structure of A. *iberus* in the north-east of the Iberian Peninsula in late spring (i.e., when this species finishes the first period of annual reproduction). Specifically, we assessed whether abiotic factors (i.e., conductivity, nutrient concentrations and pond morphology); the ponds' ecological status; food resource availability (zooplankton biomass) and the presence of the main competitor, *Gambusia holbrooki*, are correlated with the size structure and abundance of this endangered species in 10 coastal brackish and hypersaline ponds. We assessed the size structure using the size diversity index (Quintana et al., 2008), in addition to several size metrics, such as the maximum size, mean size and size range.

We hypothesized that a good ecological status, together with a larger pond dimension (depth and area), would increase the possibilities to find well size-structured populations with a higher size diversity, as well as higher densities of fish, as these two factors are supposed to set good conditions for the fish growth. Concerning conductivity, we expected a decrease in the size-related variables of *A. iberus*, because the high conductivity negatively affects its metabolism. In contrast, locations at higher conductivity levels could host higher *A. iberus* densities, because high conductivity may prevent the colonization of invasive species, such as *G. holbrooki*. We also hypothesized that the presence of *G. holbrooki* (main competitor of *A. iberus*) would lead to lower densities of *A. iberus*, as *G. holbrooki* have been observed to outcompete *A. iberus* (Alcaraz, Pou-Rovira, et al., 2008). Finally, we would expect that the abundance of *A. iberus* would be negatively correlated with the zooplankton biomass due to fish predation on zooplankton.

5.3 MATERIALS & METHODS

5.3.1 Field Sampling and Analysis

The 10 ponds studied were sampled once during the end of the spring season (from May to early June 2016). Conductivity ($mS\cdot cm^{-1}$) was measured using a multiparameter probe (Hanna Instruments, Woonsocket, RI, USA). Total area (m^2) of each pond was estimated by using the "Google Maps Area Calculator Tool" (Daft Logic, 2016), while the mean water column depth (cm) was calculated from in situ repeated measures obtained with a two-meter rule. Total nitrogen ($mg\cdot L^{-1}$) was measured according to Koroleff, 1973 (Sen Gupta & Koroleff, 1973), adapted by Seal Analytical to an integrated system of a CFA (Continuous Flow Analysis) digester.

Two ecological indices related to the pond's ecological status were used in each pond: (1) the *ECELS* (Conservation Status of Lentic and Shallow Ecosystems) estimates the conservation status of lentic ecosystems based on morphological aspects, type of aquatic vegetation and human impacts (Sala et al., 2004). The *ECELS* categories range from bad (0–30 out of 100), deficient (30–50 out of 100), mediocre (50–70 out of 100), good (70–90 out of 100) and very good (90–100 out of 100), and (2) the *QAELS* (Water Quality of Lentic and Shallow Ecosystems) index evaluates the water quality based on the composition of microcrustacean assemblages and taxonomic richness of aquatic insects and crustaceans in the Mediterranean wetlands (Boix et al., 2005). The *QAELS* categories range from bad (<0.46), deficient (0.46–0.55), mediocre (0.55–0.62), good (0.62–0.72) and very good (≥ 0.72). The *QAELS* index was calculated after the observation of macroinvertebrate samples under optic microscope and a stereoscope. Samples were obtained through a dip net (mesh size 250 μm) following standard protocols (Boix et al., 2005; Quintana, Cañedo-Argüelles, et al., 2015).

Zooplankton samples were taken from each pond by mixing subsamples from five different sites in order to overcome the expected patchy distribution of plankton. Five liters of mixed water samples were filtered through a 50-μm mesh size net and preserved in 4% Lugol's acid solution. Zooplankton individuals collected (including rotifers, copepods and cladocerans) were counted, identified (in most of cases at species level) and measured using a stereoscope and an inverted microscope (Utermöhl method), as was described in (Sgarzi et al., 2019). To estimate the zooplankton biomass, the total length (μm) of the first 100 individuals (when possible) was measured assuming that all individuals were equally distributed in the observed sample. Individual biomasses were then calculated using approximation to shape formulas.

Fish were caught by fyke nets set for 24 h, a common and widely used method in coastal lagoons (Blanco et al., 2003; Franco et al., 2012; Prado et al., 2017). Fyke nets consisted of a semicircular entrance ring followed by three smaller circular rings surrounded by a net (3.5-mm mesh) with two consecutive funnels (120 mm of funnel diameter, 1050 cm^2 of interception area, 98 cm of length, 30 cm of height and 95 cm of wing length). The total number of fyke nets set in each pond varied according to its area and depth. A total of 49 fyke nets were set in all the ponds. All captured fish were sexed (except juveniles < 13 mm), measured for total length (mm) and released. We measured all the individuals of *Aphanius iberus* in each sample, in order to minimize the error estimation.

5.3.2. *Aphanius iberus* Abundance and Size Structure

In each pond, the *Aphanius iberus* abundance was calculated by dividing the total captures by the number of fyke nets set in each pond (captures per unit of effort; CPUE). The size structure of *A. iberus* in each fyke net was assessed using four size-based metrics: (1) the maximum size, (2) mean size (computed as the geometric mean), (3) size range (the difference between the maximum and the minimum size) and (4) size diversity index (μ). For each fyke net, the size diversity was calculated using individual size (i.e., length) measurements, as proposed by (Quintana et al., 2008). Size diversity is based on the Shannon–Wiener diversity index (Pielou, 1969) adapted for a continuous variable, such as body size. This index is the continuous analog of the taxonomic Shannon–Wiener diversity index, and it produces values in a similar range to those of the Shannon–Wiener index. In our case, it integrates the amplitude of the length range and relative abundance of the different lengths. Thus, the high values of the size diversity would indicate a high diversity of sizes with an equitable numerical frequency of sizes along the distributions (Brucet et al., 2017, 2018). In contrast, the low values of size diversity (rarely taking negative values) would indicate a low diversity of fish sizes with an inequitable numerical frequency of sizes along the distribution (Quintana et al., 2008).

5.3.3 Data Analysis

We used mixed linear models (MLMs) to test the effects of abiotic and biotic factors on the abundance and size metrics of *A. iberus*. We considered captures of each fyke net as an observation unit (N = 49), and "pond" was introduced as a random effect to deal with pseudoreplication. As predictor variables, we considered conductivity, pond area and mean water depth, total nitrogen, *ECELS* and *QAELS* indexes (as estimates of ecological status) and zooplankton biomass (as food resource availability). Pearson's r index revealed correlations among some of those variables. We applied Bonferroni correction to counteract the multiple comparisons issue and, finally, removed the variables that were highly correlated (>0.6). As *G. holbrooki* was not present in all the ponds (it was absent in 7 ponds out of 10), we could not include its abundance in our MLMs. Instead, we performed an ANOVA, comparing the situations "with" and "without" the presence of *G. holbrooki* for the abundance and for each of the size-related variables of *A. iberus* considered in this study.

We looked for the most parsimonious model from the full models by using a stepwise (backward) selection. The most parsimonious model was chosen using the Akaike information criteria (lowest AIC), which represents the best at explaining the data with the lowest combination of variables. We also calculated the standardized (beta) coefficients for the significant predictors included in the best models by using the R package "QuantPsyc" version 1.5 (Fletcher, 2012). Predictors were previously checked for normality and homogeneity of variance, and, if variables did not meet the assumptions, base 10 logarithmic transformations were applied. Additionally, a visual inspection of the residual plots was done to detect any violation of the regression assumptions. In order to improve homoscedasticity, we used the function "varPower" of the package "nlme" (Pinheiro & Bates, 2000). For the creation of the boxplots, we used the "ggplot2" package (Wickham, 2016). All analyses were done with the software R version 3.4.2 (R core Team, 2017, Boston, MA, USA).

5.4 RESULTS

5.4.1 Description of the Local Characteristics in the Mediterranean Ponds

There were wide ranges of environmental and ecological conditions across the study area (Table 5). Abiotic and biotic factors measured, as well as *ECELS* and *QAELS* indexes of ecological status, are shown in Table 5. The 10 studied ponds during spring showed conductivity values ranging from 10.7 $mS \cdot cm^{-1}$ to 69.10 $mS \cdot cm^{-1}$ and mean water column depths ranging from 16 cm up to 150 cm. The ponds differed quite a lot in their areas, with values ranging from 147.90 m^2 to 68,150 m^2. Total nitrogen, which includes organic and inorganic nitrogen compounds, showed the lowest value at 55.58 $\mu mol \cdot L^{-1}$ and the highest value at 234.40 $\mu mol \cdot L^{-1}$. The zooplankton biomass ranged from 1.13 $\mu g \cdot L^{-1}$ up to 4840.48 $\mu g \cdot L^{-1}$. Regarding the ponds' ecological status, *ECELS* index values ranged from 43 to 98, indicating a "bad" and "mediocre" status, respectively. Whereas the *QAELS* index ranged from 0.25 to 0.56, also indicating a "bad" and a "mediocre" status, respectively. As an average, ponds showed a "deficient" status according to the mean index value.

Table 5. Mean, standard deviation (SD), minimum and maximum values of the abiotic and biotic factors and ecological status indexes measured in the study ponds (N = 10). *ECELS*: Conservation Status of Lentic and Shallow Ecosystems and *QAELS:* Water Quality of Lentic and Shallow Ecosystems.

	Mean	**SD**	**Minimum**	**Maximum**
Mean water column depth (cm)	59.30	41.93	16.00	150.00
Pond area (m^2)	10,873.60	20,463.50	147.90	68,150.00
Conductivity ($mS \cdot cm^{-1}$)	46.14	17.63	10.07	69.10
Total nitrogen ($\mu mol \cdot L^{-1}$)	92.01	51.63	58.55	234.40
Zooplakton biomass (µg/l)	498.17	1313.92	1,13	4840.48
ECELS index	77.00	18.70	43.00	98.00
QAELS^e 2010 index	0.47	0.12	0.25	0.56

5.4.2 Variation of the Population Structure of *A. iberus* across Mediterranean Ponds

The mean, standard deviation and minimum and maximum values calculated for the different size metrics of *A. iberus*, as well as the abundance (expressed as CPUE), are shown in Table 6. Maximum length values ranged from 19 mm to 54 mm, while the mean length had a minimum of 16 mm and a maximum of 41 mm. This last value coincided with the maximum value of the length range, whereas the minimum length range value was 6 mm. Size diversity showed a wide range of values, from 0.27 to 2.34. *A. iberus* abundance (in CPUE) also varied largely among fyke nets, from two individuals to 525 individuals.

Table 6. *A. iberus* size metrics and abundance (CPUE) obtained per each sample (N = 49). The descriptive statistics are the mean, standard deviation (SD), minimum and maximum.

	Mean	SD	Minimum	Maximum
Aphanius iberus maximum length (mm)	41.08	8.06	19.00	54.00
Aphanius iberus mean length (mm)	29.21	5.36	16.00	41.00
Aphanius iberus length range (mm)	21.67	7.38	6.00	41.00
Aphanius iberus size diversity	1.15	0.36	0.27	2.34
Aphanius iberus capture per effort unit (CPUE)	59.49	91.68	2.00	525.00

5.4.3 Main Drivers Affecting the Fish Population Size Structure and Density

The MLMs identified the most important drivers influencing the fish population size structure and density across all ponds. The most parsimonious significant models for each fish metric mentioned above (Table 6 and see the full models in Table S3 of Supporting Information) as dependent variables are shown in Table 7. The results showed that the maximum length was negatively related to the conductivity and zooplankton biomass but positively related to the total nitrogen and *QAELS* index. The zooplankton biomass was the predictor with the strongest effect on the maximum length of *A. iberus* (Table 7).

Concerning the mean length of the fish, the MLM model showed similar results as when considering the maximum length of *A. iberus* as a response variable. The mean length of *A. iberus* significantly decreased with the increasing conductivity and zooplankton biomass but showed a positive relation with the ecological quality index *QAELS*.

The length range of *A. iberus* was found to be positively related to the pond mean depth and total nitrogen. Beta coefficients of this model (0.42 and 0.48) showed similar effects of both predictor variables on the length range. The same results were found for fish density, with the CPUE positively related to the mean depth and total nitrogen (Table 7), suggesting that a higher number of individuals inhabited more nutrient-rich and larger ponds. However, the pond mean depth exhibited a stronger correlation on the fish density than the total nitrogen (beta coefficients of 0.55 and 0.36, respectively; Table 7).

Concerning the size diversity, it was positively related to the total nitrogen and only slightly related with the conductivity (Table 7). In this case, the beta coefficients for the two main drivers were similar (0.29 for conductivity and 0.30 for total nitrogen; Table 7). Finally, the *ECELS* index and pond area were the only variables not retained in any of the models selected (see the full models in Supplementary Table S3).

Table 7. Results of the linear mixed models (N = 49 fyke nets) showing the predictor variables that significantly relate with *Aphanius iberus* size metrics and abundance (expressed as CPUE). Only the most parsimonious significant models were shown for each response variable. For each model, the intercept (estimated and standard error, S.E.), beta coefficients (standardized), *t*-value, significance (*p*-value) and degrees of freedom (df) are also reported.

Response variable	Predictor	Estimate	S.E.	Beta coefficients	*t*-value	*p*-value	df
Aphanius iberus MAXIMUM length	Conductivity	-19.49	4.69	-0.47	-4.15	<0.01	5
	Log Total Nitrogen	2325.09	564.20	0.45	4.12	<0.01	5
	QAELS index	3894.26	690.27	0.59	5.64	<0.01	5
	Zooplankton biomass	-0.22	0.05	-0.69	-4.51	<0.01	5
Aphanius iberus MEAN length	Conductivity	-0.15	0.05	-0.42	-3.35	0.02	6
	QAELS index	31.94	6.76	0.55	4.73	<0.01	6
	Zooplankton biomass	-0.01	-0.01	-0.59	-4.01	0.01	6
Aphanius iberus length RANGE	Log Pond Mean Depth	14.45	5.36	0.42	2.70	0.03	7
	Log Total Nitrogen	36.88	8.93	0.48	4.13	<0.01	7
Aphanius iberus SIZE DIVERSITY	Conductivity	<0.01	<0.01	0.29	2.21	0.06	7
	Log Total Nitrogen	0.93	0.37	0.30	2.51	0.04	7
Aphanius iberus capture per effort unit (CPUE)	Log Pond Mean Depth	1.46	0.57	0.55	2.56	0.04	7
	Log Total Nitrogen	2.15	0.97	0.36	2.23	0.06	7

5.4.4 Influence of the Presence of *G. holbrooki* on the Size Structure and Density of *A. iberus*

Boxplots showed the CPUE and size metrics of *A. iberus* in the presence and absence of *G. holbrooki* in the pond (Figure 9). Overall, the presence of *G. holbrooki* in the pond did not significantly modify the size structure and density of *A. iberus* (*p*-values > 0.39; Figure 9). However, the mean size of *A. iberus* was significantly higher when *G. holbrooki* was present in the pond (Figure 9), indicating an unexpected increase of body size with the presence of the main competitor.

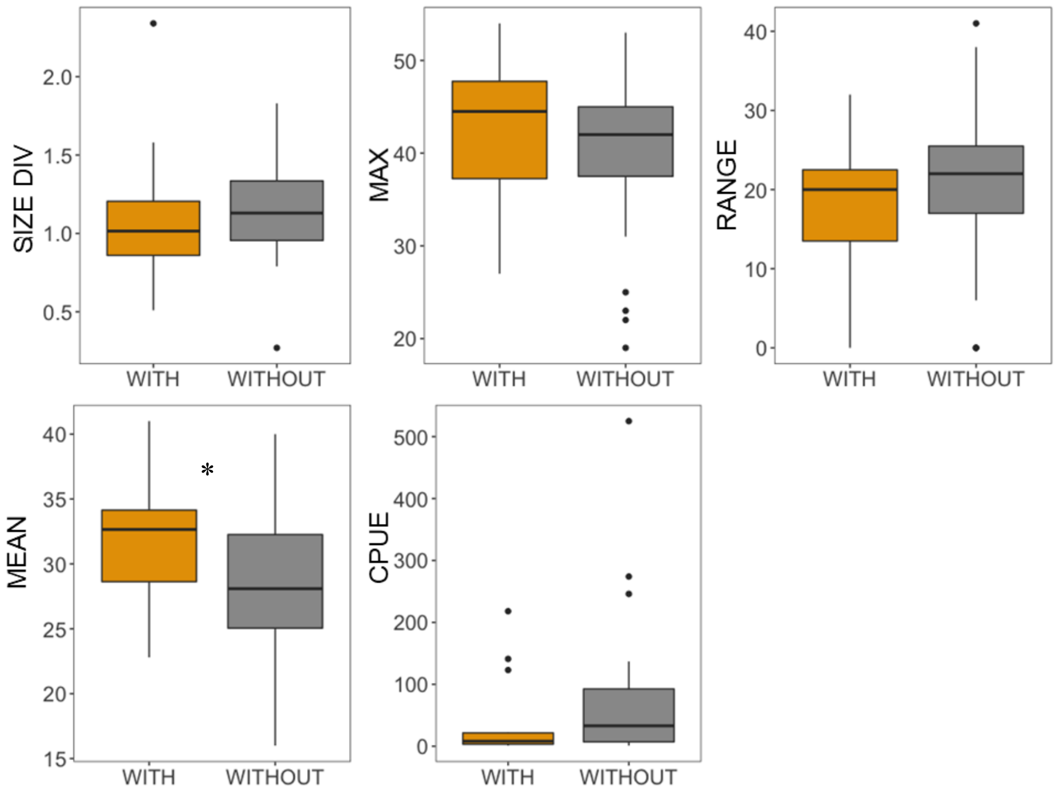

Figure 9. Boxplots showing the distribution of *Aphanius iberus* capture per unit of effort (CPUE) and size-related variables according to the presence ("WITH") or absence ("WITHOUT") of *Gambusia holbrooki*. Significant differences are marked with the asterisk symbol (*).

For a graphical visualization of the results obtained, see Figure S5 in Supporting Information.

5.5 DISCUSSION

Our results suggested that both the maximum and mean sizes of *A. iberus* increased with the increasing pond's water quality (*QAELS* index) and decreased with the increasing conductivity and zooplankton biomass. The size range, maximum size, size diversity and CPUE of *A. iberus* were positively related to the nutrient concentration (i.e., total nitrogen), while the size range and CPUE were also larger in deeper ponds. In contrast to our hypothesis, the presence of *G. holbrooki* seemed not to affect negatively the population structure of *A. iberus*.

We found larger maximum and mean lengths of *A. iberus* in locations with better water quality (i.e., the *QAELS* index). These results support a previous study on multiple water bodies in the southernmost distribution area of *A. iberus*, showing its preference for ponds of better ecological status (Casas et al., 2011). Although the results showed that the average of the studied locations had a deficient ecological status, higher values of the *QAELS* index are usually associated with the predominance of large zooplankton (such as big copepods) over small rotifers (that are more linked to eutrophic and hypoxic conditions; (Blanco et al., 2003; Brucet, Boix, et al., 2005). Adults of *A. iberus* (with larger body lengths) are usually associated with glasswort habitats (highly productive and occasionally inundated environments) where big zooplankton is more abundant, and this may support the positive relationship between *A. iberus* size and the ecological status of the ponds found in our study. In contrast, younger and smaller individuals positively select more eutrophic algal mats, associated with a bad ecological status, where small rotifers dominate (Alcaraz & García-Berthou, 2007).

The total nitrogen was found to be related with the CPUE, as well as with all size metrics, expect the mean length. In Mediterranean salt marshes, the concentration of total nitrogen in the water is an indicator of a pond's confinement or isolation (Quintana et al., 1998), and during the late-spring and summer season, the total nitrogen is more concentrated because of evaporation processes (Badosa et al., 2006). Our results are in accordance with previous studies that showed that, in more confined and less accessible ponds, the populations of *A. iberus* are more abundant and stable over time, probably due to a lack of external perturbations, such as isolation from invasive species (Prado et al., 2017) or fewer entries of freshwater inputs. Since nitrogen is typically the limiting nutrient in these ponds, a higher total nitrogen concentration could also be associated with higher production rates of *A. iberus*, which, in turn, may favor larger populations.

Our results also showed, as expected, a negative relationship among conductivity and the maximum and mean sizes of *A. iberus*. In high saline habitats, conductivity can act as a "refuge" for the *A. iberus* to avoid the colonization of less salt-tolerant fish species, such as *G. holbrooki* (Leonardos & Sinis, 1998). However, high salinity levels may also have negative effects on the metabolism of cyprinodontoids (Oliva-Paterna et al., 2006; Plaut, 2000), because the energy used for osmoregulation is not available for their growth performance and survival (Brett & Groves, 1979; Wootton, 1989; Yildirim & Karacuha, 2008). This could explain the presence of smaller fish in ponds at higher levels of conductivity.

In our study, the *A. iberus* CPUE, along with the *A. iberus* size range, were positively related to the pond depth. Similarly, studies from European wetlands and lakes found that wider and deeper waterbodies hosted greater biomass and sizes of fish (Prado et al., 2017; Søndergaard et al., 2005), with the consequent higher probability to find a wider range of fish sizes (Arranz et al., 2016). Additionally, individuals of *A. iberus* trapped in brackish ponds due to competition exclusion and habitat degradation can, in some cases, reach unnaturally high densities (Prado et al., 2017).

Our results also showed that the zooplankton biomass is negatively correlated to the *A. iberus* maximum and mean sizes. Another study on *A. iberus* observed that both juveniles and adults of this species have similar food preferences, as they mainly feed on harpacticoids, copepods and nauplii, detritus and diptera larvae (Alcaraz & García-Berthou, 2007). Still, smaller individuals prefer feeding on small-sized prey, while larger fish show a greater preference for large-sized prey (Alcaraz & García-Berthou, 2007). Larger individuals have higher feeding rates (Peters & Downing, 1984; Wootton, 1989). Thus, the presence of larger fish (expressed by higher mean and maximum sizes) may imply a lower zooplankton biomass, as it increases the consumption rates with the fish body sizes. In addition, previous studies observed that, when the potential resource availability is low, the fish size distribution tends to be more diverse, suggesting that competitive interactions for resources promote diversification by size (Brucet et al., 2006; Emmrich et al., 2011; Quintana, Arim, et al., 2015).

As for the influence of *G. holbrooki* on *A. iberus* abundance and size structure, the results suggested that both the CPUE and size metrics of *A. iberus* were not affected by the presence of this allochthone fish. Only the mean size seemed to be slightly positively affected by the presence of the competitor. This result differed from our expectations, in which *A. iberus* would be smaller and less abundant in the presence of *G. holbrooki*. This apparent inconsistency could be explained by the fact that *G. holbrooki* was found just in few of the studied ponds, the ones with lower conductivity, and what we observed could be an indirect effect of environmental conditions that favor *A. iberus* development more than the effect of direct competition. Thus, the low number of ponds with *G. holbrooki* in our study did not unable us to derive strong conclusions about the influence of the *G. holbrooki* presence on *A. iberus* abundance and size structure.

In conclusion, our results suggest that the ponds' ecological status (as shown by the *QAELS* index), depth, conductivity and nutrient concentrations are key variables that determine the variations of the size structure and abundance of *A. iberus* in Mediterranean brackish ponds. Achieving a better pond ecological status seems to be important for the conservation of endangered *A. iberus,* because better size-structured populations (i.e., larger mean and average lengths) are found at higher water quality conditions. In addition, a pond's isolation may also be an advantage to preserve *A. iberus* populations.

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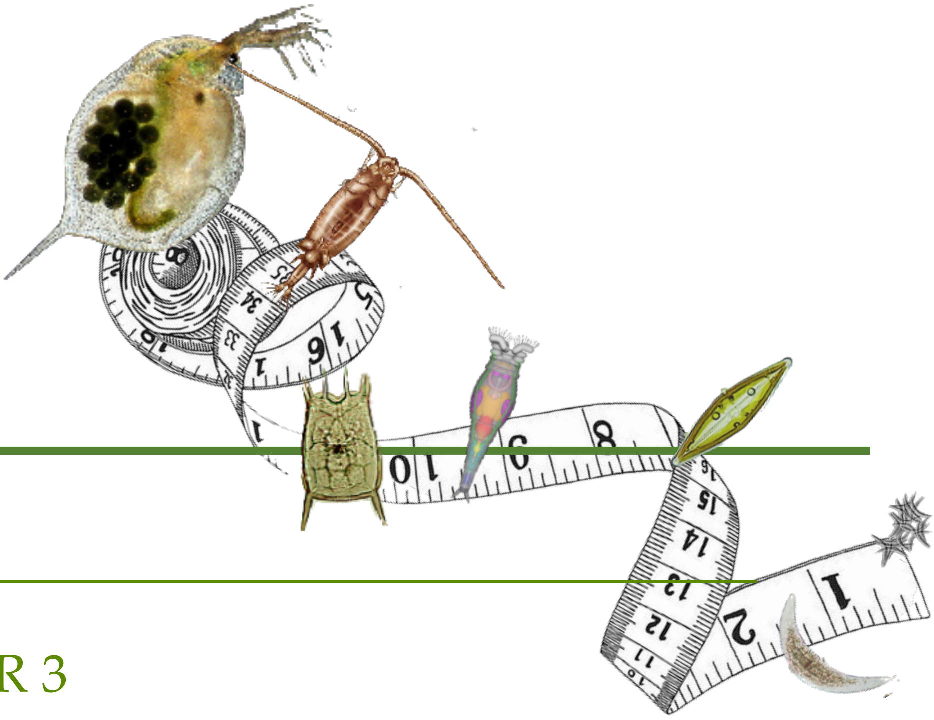

6. CHAPTER 3

Water depth and resource availability mediate planktonic size spectra in temporary Mediterranean ponds along the hydroperiod

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6.1 ABSTRACT

Mediterranean temporary ponds are shallow ponds that undergo a periodic cycle of flooding and drought. These ponds are ecologically important ecosystems, supporting many endemic and rare species. In the current study, we have focused on three freshwater temporary ponds located in NE Spain, monthly sampled for three consecutive hydroperiods from 2016 to 2019. The length of the hydroperiod varied among the ponds and the sampling year, ranging from 2 to 6 months. The parameters of the size spectra (i.e., negative linear relationship between individual body size and abundance) were analysed for phytoplankton, zooplankton, and both phytoplankton and zooplankton communities together in relation to temporal variability of environmental variables and predation pressure. We hypothesized that the shape of the size spectra would change as a response to the environmental variations along the hydrological cycle, and it would reflect changes in resource availability and in the predation pressure. In general, results showed that an increase in resource availability and a decrease of water column depth leads to an increase in the size spectra intercepts of all planktonic groups, reflecting an increase in abundance of individuals in all the size classes. Also, as mean water column depth decreased along the hydroperiod, the slope of the whole planktonic size spectrum flattened (indicating an increase in the relative abundance of large individuals). In contrast to our expectations, size spectrum parameters did not respond significantly to predation neither for phytoplankton nor zooplankton communities. Results of the present study showed that in temporary Mediterranean ponds the size spectra of planktonic communities reflect the systematic changes of organisms' abundances and sizes (overall increase in total abundance and in large size individuals) that occur along the hydroperiod.

Key words: Mediterranean temporary ponds, size spectrum, trophic chain, phytoplankton, zooplankton, macroinvertebrates.

6.2 INTRODUCTION

Temporary ponds in the Mediterranean region are unique ecosystems that have high biodiversity (Bagella et al., 2016) and are home to rare and endangered species of plants and invertebrates (Zacharias & Zamparas, 2010). Besides biodiversity, these environments provide important ecosystem services, such as nutrients retention and hydrological regulation (Céréghino et al., 2008), the improvement of water quality, the sequestration of CO_2 from the atmosphere (Zedler & Kercher, 2005), in addition to their aesthetic and landscape value. In general, temporary water bodies are highly vulnerable to anthropic impacts (e.g., agriculture, urbanization, eutrophication, increased salinity, etc.) due to their temporary and shallow nature (Rhazi et al., 2004). As a result, temporary ponds are disappearing at an alarming rate around the world (Zacharias & Zamparas, 2010). Currently, climate change and water scarcity add an extra pressure to the conservation of Mediterranean temporary ponds (Zacharias & Zamparas, 2010). Due to their singularities and their high flora and fauna richness, Mediterranean temporary ponds are considered habitats of priority interest by the Habitats Directive (Council Directive 92/43/EEC) of the European Union and are part of the 'Natura 2000 Network but were excluded from the European Water Framework Directive due to their small size (Kristensen & Globevnik, 2014; Van Den Broeck et al., 2015).

Most ecological studies of aquatic communities in Mediterranean temporary ponds have been focused on their taxonomy (Boix et al., 2001; Ghosn et al., 2010; Lumbreras et al., 2016), and there are a few studies focusing on the size structure of their aquatic communities (Boix et al., 2004; Brucet, Boix, et al., 2005; Quintana et al., 2006). Several studies in aquatic ecosystems show that community size structure responds to environmental changes and gives also crucial information about predator-prey interactions and trophic transfer efficiency along the trophic chain (Ahrens & Peters, 1991; Brucet, Boix, et al., 2005; Sprules & Munawar, 1986; Zhang et al., 2013). One way to analyse the size structure of a community is through the size spectrum (Kerr & Dickie, 2001). The size spectrum represents the inverse linear relationship between the abundance of organisms and the individual body size, as a result of the faster metabolism of small-sized organisms (Platt & Denman, 1977; Sprules & Munawar, 1986) and the loss of energy within and among trophic levels (Brown et al., 2004). Size spectrum can be constructed considering a single community or trophic level (i.e., ecological size spectrum; (Brucet, Boix, et al., 2005; Dickie, 1987)), or the whole food web (i.e., physiological spectrum; (Sprules & Barth, 2016)). The former gives mainly information about biotic relations within a trophic level (e.g., predation, competition), whereas the latter gives information about the biotic relationships between the different trophic levels and about the trophic transfer efficiency (TTE, the ratio of production rates between adjacent trophic levels) of the food web (Brown et al., 2004; Brucet et al., 2018; Treblico et al., 2013). The parameters of the size spectrum (i.e., linear relationship) are the slope and the intercept. The slope indicates the relative contribution of small-sized and large-sized individuals and is an indicator of the trophic transfer efficiency (Mehner et al., 2018). The size spectrum slope has a theoretical value across ecosystems and organisms in steady state conditions (e.g., −2 in Normalized Abundance Size Spectra) (Polishchuk & Blanchard, 2019), but it consistently varies in response to environmental gradients (Brucet et al., 2013; Emmrich et al., 2011), anthropogenic pressures (Jennings & Blanchard, 2004; Pomeranz et al., 2019), and biotic factors (Arranz et al., 2016; Jinks et al., 2019). Steeper slopes represent an increase in the relative abundance of smaller individuals and vice versa. Concretely, flatter slopes represent an increase in the TTE (which normally is around 10%) (Mehner et al., 2018). Several studies observed flatter size spectrum slopes (i.e., higher relative abundance of large individuals) as a response to increase nutrient availability (Gaedke et al., 2004; Sprules & Barth, 2016; Sprules & Munawar, 1986; Tittel et al., 1998).

The size spectrum intercept may be interpreted as an estimation of the food-web capacity or productivity potential (i.e., biomass or number of individuals supported by the food web (Boudreau & Dickie, 1992; Gaedke, 1992; Sprules & Munawar, 1986). The intercept, reflecting carrying capacity or abundance, can be modified by abiotic factors, such as productivity (Benejam et al., 2018; Boudreau & Dickie, 1992), temperature (Boudreau & Dickie, 1992), nutrients circulation and availability (Boudreau & Dickie, 1992; Murry & Farrell, 2014). A rise in the carrying capacity is generally associated with increasing productivity and nutrient inputs (Ahrens & Peters, 1991; Boudreau & Dickie, 1992; Sprules & Munawar, 1986; Zhang et al., 2013). However, trophic interactions such as predation and competition are also important drivers of the community abundance (Murry & Farrell, 2014; Shin et al., 2005; Zimmer et al., 2001). Predation pressure (mainly exerted by fish in permanent water bodies), often leads to a decrease in relative abundance of large-sized organisms in the adjacent trophic level, resulting in a steeper size spectrum slope (Almond et al., 1996; Brucet, Boix, et al., 2005), and, less frequently, a decrease in the size spectrum intercept (Murry & Farrell, 2014; Shin et al., 2005; Zimmer et al., 2001).

In temporary ponds, community structure changes as hydroperiod progresses and the habitat dries, along with changes in water chemistry, and the decreasing of water depth (Boix et al., 2004; Florencio et al., 2014; Williams, 1997). In temporary ponds, predation pressure exerted by large macroinvertebrates on smaller individuals (i.e., zooplankton) can lead to a flattening of the size spectrum (including zooplankton and macroinvertebrates) as abundance of large macroinvertebrates increased along the hydroperiod (Boix et al., 2004). A flattening of the zooplankton size spectrum slope along the hydroperiod was also observed in brackish temporary ponds as a result of trophic interactions (Brucet, Boix, et al., 2005). However, the studies that have evaluated how organisms' size structure changes along time in temporary ponds are very scarce (Boix et al., 2004; Brucet, Boix, et al., 2005). The examination of the size spectra of the communities of temporary ponds along the hydroperiod may bring insight into the abiotic and biotic variables influencing the different trophic levels and into the biomass transfer through the food web (Braun et al., 2021; Brucet, Boix, et al., 2005; García-Comas et al., 2016; Mehner et al., 2016).

The present study aimed (1) to model the size spectrum of the plankton community along the hydroperiod in Mediterranean temporary ponds using both ecological scaling (phyto- and zooplankton groups for separate) and physiological scaling (both groups together), and (2) to analyse the influence of environmental variables, predation, and food resource availability on the size spectrum parameters. We hypothesized an increase of spectrum intercepts (i.e., abundances) and a flattening (i.e., less negative) of size spectrum slopes (i.e., higher relative abundances of larger individuals) of both phytoplankton and zooplankton along the hydroperiod (i.e., as water level decreases). As a result of predation effects on phytoplankton and zooplankton we expect a negative relationship between the predator biomass (i.e., zooplankton and macroinvertebrates, respectively) and the size spectrum intercept and slope of prey (i.e., phytoplankton and zooplankton). This means lower intercepts and steeper slopes under high predation pressure.

6.3 MATERIALS & METHODS

6.3.1 Study area

The study was developed in the Can Torres estate in the Albera Mountain Range, a natural protected area of National Interest, located in the NE of Catalonia region (NE of Iberian Peninsula). In this estate there are several environments of natural interest such as lowlands hay meadows and three temporary ponds: “La Rajoleria”, “La Cardonera” and “Prats del Roser”. As typical of Mediterranean climate, weather in this area is defined by hot and dry summers, and mild and wet winters with a mean annual precipitation of 582 mm and a mean annual temperature of 14.9 °C, respectively (Meteorological Service of Catalonia, 2013). The hydrological pattern of the studied temporary ponds is characterized by a periodic cycle of flooding and drought. The absence of groundwater inputs and the isolation from other water bodies imply that the hydroperiod length depends entirely on precipitations and external temperature (Ruhí et al., 2014). The flooding phase usually coincides with autumn or spring rainfalls (depending on annual variations) whereas drought usually occurs in summer. Previous studies (Sgarzi et al., 2017, 2018, 2019, unpublished data) showed that the studied ponds have a good water quality according the *QAELS* index (acronym of ‘water quality of lentic shallow environments’ in Catalan) developed for lentic shallow ecosystems in Catalonia and based on the aquatic invertebrate community (Boix et al., 2005).

6.3.2 Sampling & Analysis

The three ponds were sampled monthly during three consecutive hydroperiods (i.e., years) with different lengths for each pond. The first hydroperiod lasted from December 2016 to May 2017, the second hydroperiod lasted from May to June 2018, and the third from November 2018 to March 2019. Physicochemical variables such as conductivity ($\mu S \cdot cm^{-1}$), temperature (°C) and dissolved oxygen (%) were measured using a multiparameter probe (Hanna Instruments, Woonsocket, RI, USA). Mean water column depth (cm) was calculated from *in-situ* repeated measures obtained with a two-meter rule. Total nitrogen ($\mu mol \cdot L^{-1}$), total phosphorous ($\mu mol \cdot L^{-1}$) and nitrates ($\mu mol \cdot L^{-1}$) were measured according to (Sen Gupta & Koroleff, 1973) adapted by Seal Analytical to an integrated system of a CFA (Continuous Flow Analysis) digester. Chlorophyll-*a* (μg·L^{-1}) was measured using spectrophotometry after 90% acetone extraction following (Parsons & Strickland, 1963).

Water samples for planktonic organisms were taken along the water column, collecting at least 10L from three different points and subsequently mixing in a bucket to overcome the expected patchy distribution of plankton. Zooplankton samples (including rotifers, copepods and cladocerans) were obtained by filtering 5 to 15 L (depending on the abundance) through a 50 μm mesh size net and phytoplankton samples were obtained by collecting 125 ml of unfiltered water. Both samples were preserved in 4% acid Lugol′ solution. Macroinvertebrate samples were collected through a dip net (mesh size 250 μm) following standard protocols (Boix et al., 2005) and preserved in ethanol 70%. The water level at Prat del Roser, especially at the end of the hydroperiods, was not enough to allow the withdraw of macroinvertebrates samples according to the method specified before. Counting, identification (when possible, at species level) and measuring of aquatic organisms were performed using a stereomicroscope for larger zooplankton and macroinvertebrates and an inverted microscope for smaller zooplankton and phytoplankton (Utermöhl method). Phytoplankton was counted and measured following the protocol for phytoplankton identification described in the EU project 'WISER' (Water bodies in Europe: Integrative Systems to assess Ecological status and Recovery) (Mischke et al., 2012). Horizontal transects were counted until 100 individuals of the most abundant species had been enumerated (see Table S6, Supporting Information). For each species, at least 10 individuals were measured. For zooplankton, at least 100 individuals of the most abundant taxa were counted, and the first 25 individuals of each species were measured (when possible). For macroinvertebrates all the individuals were counted and measured.

Biomass (μg dry weight) for zooplankton (copepods and cladocerans) was estimated by using equations of the allometric relationship between body length (in μm) and biomass (μg of dry weight) (Dumont et al., 1975; Malley, et al., 1989). For phytoplankton and rotifers, biomass estimations were obtained by converting biovolume into dry weight (Yvon-Durocher et al., 2011). Biovolume was estimated through the approximation of the body shape to geometric models (Mischke et al., 2012; Ruttner-Kolisko, 1977). Total length of macroinvertebrate individuals was measured following the protocol of (Moretti et al., 2017) and biomass (mg dry weight) was estimated following body length-mass relationship formulas provided for the different taxonomic groups (Benke et al., 1999; Meyer, 1989; Smock, 1980; Traina & von Ende, 1992). As we were interested in the effect of predation from macroinvertebrates on zooplankton, for further analyses, macroinvertebrates were divided into two groups: predators and non-predators. According to the bibliography, planktivorous macroinvertebrates belonged to the following taxonomic groups: Amphipoda (fam. Niphargidae), Anostraca (genus *Chirocephalus*), Diptera (fam. Chaoboridae), Hemiptera (fam. Pleidae), Odonata (fam. Libellulidae and Lestidae), Trichoptera (fam. Hydropsichydae), Heteroptera (genus *Notonecta* and fam. Corixidae), Coleoptera (fam. Hygrobiidae and Hydrophilidae, and genus *Gyrinus* and *Berosus*) (Benke et al., 1999; Meyer, 1989; Smock, 1980; Traina & von Ende, 1992) (see Table S6, Supporting Information).

6.3.3 Size Spectra calculation

To analyse the size structure of planktonic communities, we constructed the size spectrum separately for phytoplankton and zooplankton, and the size spectrum including both trophic levels (phytoplankton and zooplankton together). Therefore, we considered both ecological spectra (i.e., within a specific functional group such as phytoplankton and zooplankton), called also ecological scaling, and physiological spectra (i.e., phytoplankton and zooplankton together) (Dickie et al., 1987). Following the binning method (Sprules & Barth, 2016; White, 2008), we grouped individual sizes into different size classes in $\log_2$ scale depending on the community: 28 size classes for phytoplankton, 23 size classes for zooplankton, and 31 classes for phytoplankton and zooplankton together. The size classes ranged from 1.19×10^{-7} to 32.00 µg for phytoplankton, from 3.05×10^{-5} to 256.00 µg for zooplankton, and from 1.19×10^{-7} to 256.00 µg for phytoplankton and zooplankton communities together. We then normalized the abundance by dividing abundances with the linear width of the bins (Sprules & Barth, 2016; White, 2008). Size spectrum parameters (slope and intercept) were then extracted using the linear regression between the $\log_2$ of midpoint of the size classes (x axis) and the $\log_2$ normalized abundance (y axis) (White, 2008). Only size spectra with a p-value < 0.05 were finally considered in the study (79 out of 81).

6.3.4 Data Analysis

We conducted a principal component analysis (PCA) to summarize variation in environmental variables (total nitrogen ($\mu mol \cdot L^{-}$), total phosphorous ($\mu mol \cdot L^{-}$), nitrates ($\mu mol \cdot L^{-}$), conductivity ($\mu S \cdot cm^{-1}$), dissolved oxygen (%), chlorophyll-*a* ($\mu g \cdot L^{-1}$), temperature (˚C) and depth (cm)) across the three ponds during the study period, using 'prcomp' function in 'stats' package (R Development Core Team, 2020). We centred and scaled all variables before performing PCA. The first axis of the PCA explained 39.9% of the variation of the environmental variables and was positively related with total nitrogen, conductivity, temperature, total phosphorous and chlorophyll-*a* (Dim1, Figure 10), whereas the second axis of PCA explained 17.3% of variation, and it was positively related with Nitrates (Dim2, Figure 10). Mean water column depth and dissolved oxygen showed a low contribution to the variation explained. It is worth noting that mean water column depth was not significantly correlated to PCA1 axis (r= -0.33, p-value= 0.09).

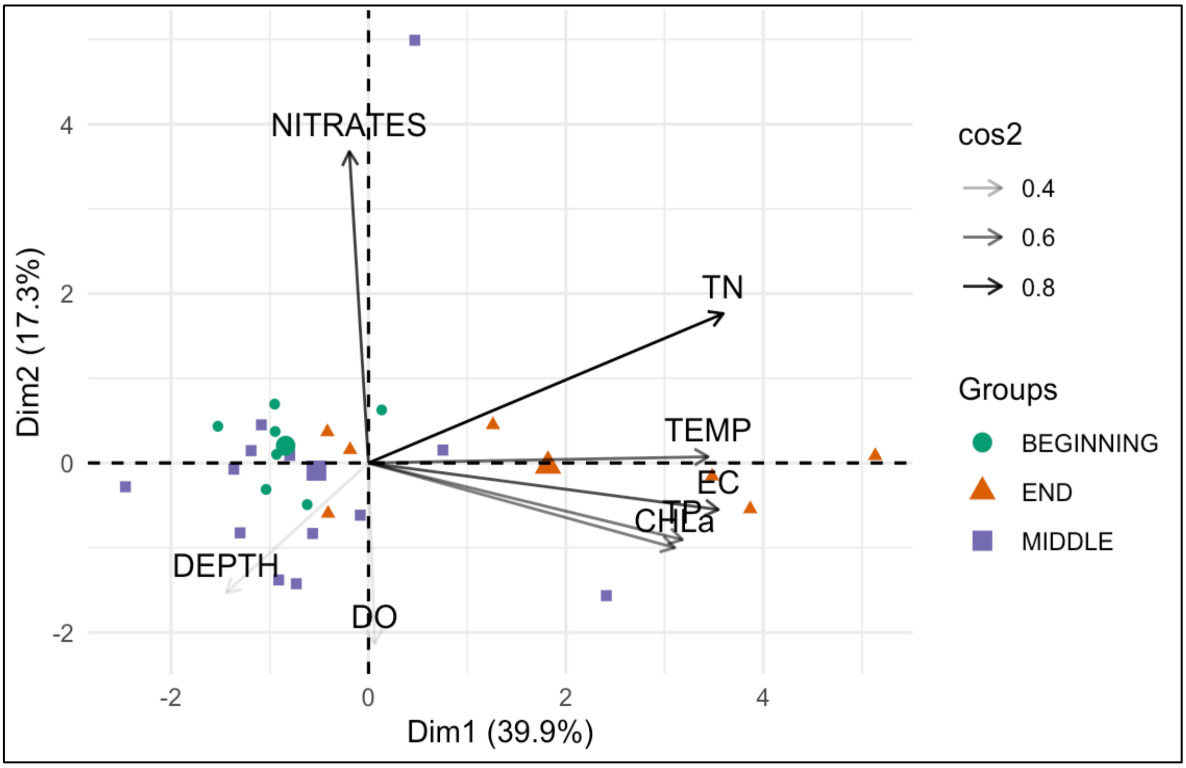


Figure 10. Principal Component Analysis (PCA) in order of contribution, to summarize variation in environmental variables (TN, total nitrogen (μmol·L); TP, total phosphorous (μmol·L); NITRATES, nitrates (μmol·L^{-1}); EC, conductivity (μS·cm^{-1}); DO, dissolved oxygen (%); CHLa, chlorophyll-*a* (μg·L^{-1}), TEMP, temperature (˚C); DEPTH, mean water column depth (cm) across the three ponds during the study period. 'cos2' (squared cosine), indicates the contribution of a variable to the squared distance of the observation to the origin (the darker is the arrow, the higher is the importance of the variable). The separation of the samplings is based on the phase of the hydroperiod, being the 'beginning' the first month of flooding, 'end' the last month of flooding, and 'middle' the months in between, considering the three ponds together.

In the subsequent statistical analyses, PCA1 axis scores were used as an independent variable to reduce the number of predictor variables in the models.

We conducted linear mixed models (LMMs) (‘nlme’ package, (Pinheiro, 2021)) to test the effects of abiotic and biotic factors on the size spectrum parameters. A total of six response variables were analysed in the present study: the slopes and the intercepts of the phytoplankton and zooplankton size spectra for separate (ecological scaling), and the phytoplankton and zooplankton together size spectra (physiological scaling). The following variables were used as fixed effect predictors: PCA1 axis, which was related to chlorophyll-*a* and total nitrogen; mean water column depth (as a proxy of hydroperiod phase); biomass of macroinvertebrate predators (as an indicator of predation pressure on zooplankton) and zooplankton biomass (as an indicator of grazing on phytoplankton) (see Table 8 for the specific relationships tested). To control for temporal and spatial variability, we used sampling date nested in pond identity as random factors in each model. The most parsimonious models were selected by using stepwise (backward) selection, using the Akaike information criteria (lowest AIC) which represents the best model at explaining the variance in the dependent variable with the fewest number of independent variables (Bozdogan, 1987). We checked the diagnostic plots of residuals of the models for the homogeneity of variance and tested the normality of residuals by Shapiro–Wilk’s test ($p > 0.05$). All the predictor variables considered were not significantly correlated among them (Pearson’s index of correlation $r<0.6$). All analyses were performed with the software R version 4.0.5 (R core Team, 2021), and all graphs were plotted using “ggplot2” R package (Wickham, 2016).

Table 8. Outline of response and predictor variables used to test for the relationships in the Linear Mixed Models.

Response variables	**Predictor variables**		**Random factors**
	Environmental	**Predation**	
Phytoplankton SS intercept & slope	PCA1 Water mean depth	Zooplankton biomass	Sampling DATE nested POND
Zooplankton SS intercept & slope	PCA1 Water mean depth	Macroinvertebrates biomass	Sampling DATE nested POND
Phytoplankton + Zooplankton SS intercept & slope	PCA1 Water mean depth	Macroinvertebrates biomass	Sampling DATE nested POND

6.4 RESULTS

6.4.1 Temporal Variations in Environmental Characteristics of the Study Ponds

Hydroperiod length was different for each pond. Rajoleria remained flooded during more months in the first and third hydroperiod (i.e., first and third year) and was the only pond which had water during the second year, although just for two months (Figure 11). The pond with the shortest hydroperiod was Prats del Roser, which was flooded for four months during the first year, and only two during the third year. Cardonera was flooded for four months in the first and in the third year. Water depth generally decreased along the hydroperiod in all ponds until the total drought, with some punctual increases in March of the first hydroperiod in Rajoleria and Cardonera due to rainfall (Figure 11). As water depth decreased, temperature tended to increase. Nitrates showed a decreasing trend at the beginning of the hydroperiod, whereas the highest levels of total nutrient concentrations (TN and TP) were found in all ponds by the end of the first hydroperiod (e.g., April and May 2017), where also the highest chlorophyll-*a* concentrations were observed (Figure 11). Oxygen increased along time in all ponds, and decreased in spring (i.e., from late April) during the first hydroperiod (Figure 11). This decrease in oxygen was not observed during the third year when the ponds dried out in earlier or late winter.

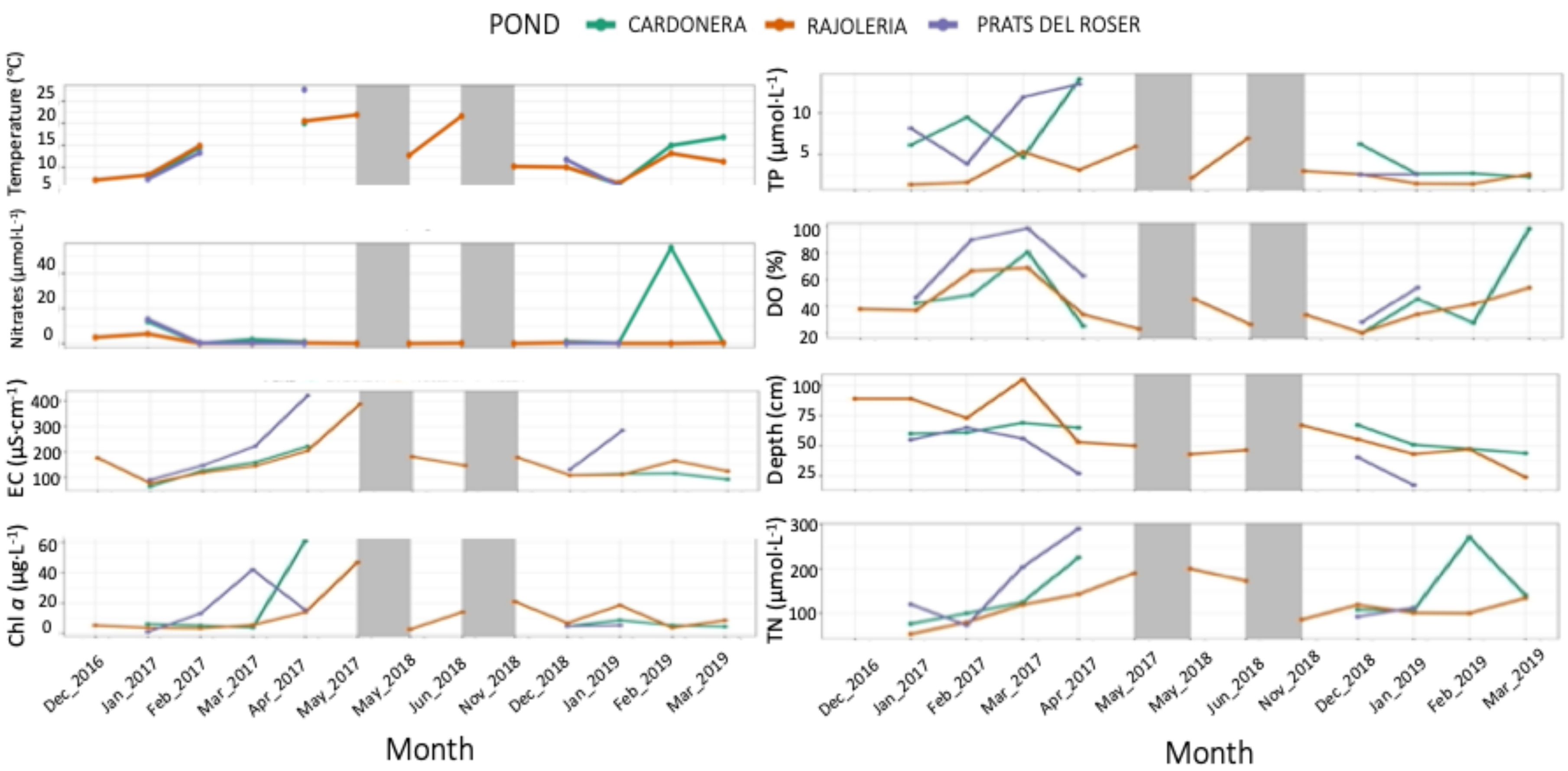


Figure 11. Temporal evolution of the environmental variables along the three hydroperiods studied (separated by the grey columns, meaning drought of all ponds) for each of the ponds (Rajoleria, Cardonera and Prats del Roser). Variables shown are Temperature (°C), Nitrates (µmol·L-1), Electrical conductivity (EC, µS·cm-1), chlorophyll-*a* (Chl *a*, µg·L-1), total phosphorous (TP, µmol·L-1), dissolved oxygen (DO, %), mean water column depth (Depth, cm) and total nitrogen (TN, µmol·L-1) evolution in time are shown for each of the ponds studied.

6.4.2 The Aquatic Communities

With regards to phytoplankton community, a total of 97 species belonging to 10 taxonomic classes were identified (Figure 12). In almost 40% of the samples, >50% of individuals were organized in colonies. The individual lengths ranged from 2.8 µm (Cyanophyceae cell), to a length of 660 µm (Chlorophycea colonies). In Rajoleria during the first hydroperiod, phytoplankton taxonomic groups did not seem to follow a clear pattern of dominance along with the hydroperiod, with exception of a general trend of Cryptophyceae decreasing until the drought (last month), happening for both first and third hydroperiods. The same pond, during the short second hydroperiod, showed the dominance of Bacillariophyceae during the first month, followed by the dominance of Cryptophyceae during the last month. Similarly, in the third hydroperiod, Bacillariophyceae dominated at the beginning, and Cryptophyceae in the middle phase. Cardonera showed the dominance of different taxonomic groups from Rajoleria, as in both first and third hydroperiods Chlorophyceae and Chrysophyceae prevailed. In the third hydroperiod the dominance of Chrysophyceae at the beginning

Figure 12. Relative abundance (%) of the main groups of phytoplankton in the 3 studied ponds along de hydroperiods (separated by thick black lines).

is replaced by the dominance of Chlorophyceae at the end. In Prats del Roser, there is a clear dominance in both hydroperiods of Dinophyceae at the beginning, and Euglenophyceae and Chlorophyceae, in first and third hydroperiods respectively during the other phases. Chlorophyceae, which usually form colonies, dominated at the end of both hydroperiods in Cardonera and in Prats del Roser (except for the first hydroperiod of Prats del Roser, where there is a lack of a clear taxonomic dominance).

Within the zooplankton community (Figure 13), rotifers were the smallest individuals (0.05 mm) and the largest were calanoid copepods (4.18 mm). Cladocera, which are known to be usually larger than copepods, in this study had a mean size of 0.7 mm. Regarding zooplankton dominance, smaller sizes such as rotifers, copepods nauplii and juveniles (copepodites) dominated over the other taxonomic groups during the three hydroperiods in all the ponds studied (Figure 13), representing, together, more than 50% of the community abundance in each sampled month. Adults of the

Figure 13. Relative abundance (%) of the main groups of zooplankton in the 3 studied ponds along de hydroperiods (separated by thick black lines). Note that for the zooplankton, larval stages of copepods (copepodites + nauplii) include all orders: calanoids, cyclopoids and harpacticoids.

three copepod orders (i.e., calanoids, cyclopoids and harpacticoids) were scarce (less than 20% of the community all together), as well as Cladocera (representing less than 20% of the community), except for Rajoleria and Prats del Roser during April 2017, where Cladocera represented about 50% of the community. Despite the dominance of smaller taxonomic groups, the relative abundance of Cladocera and adults of copepods increased over time, particularly during the longest hydroperiods.

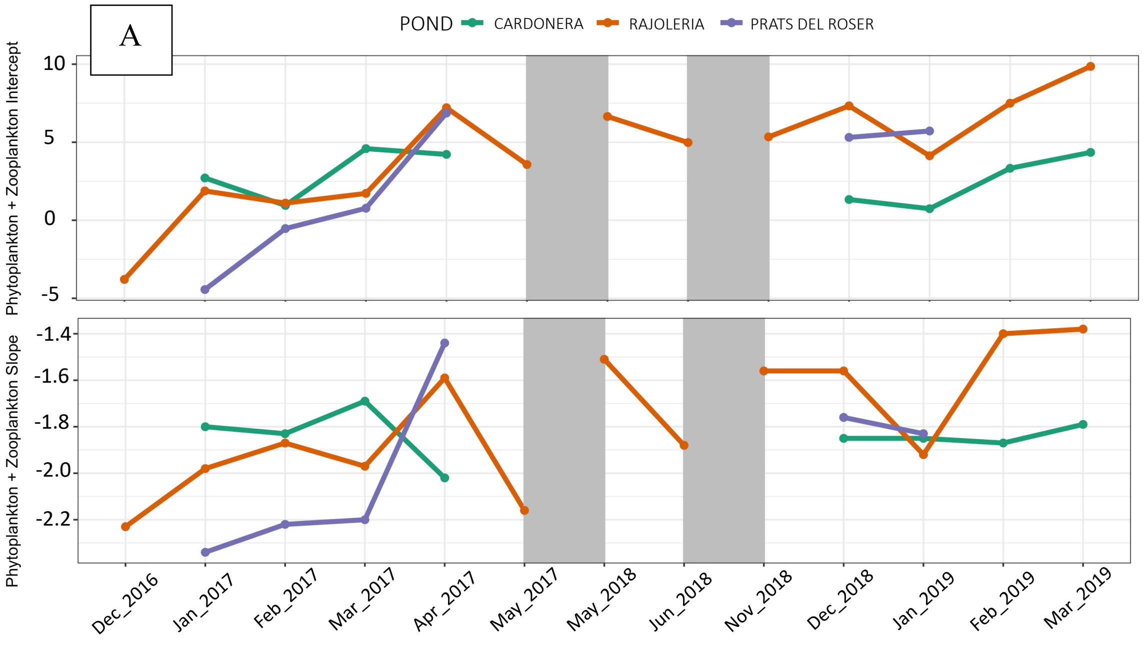
Macroinvertebrates were separated into 2 groups: zooplankton predators and non- predators (Figure 14). A dominance (more than 60% on average) of predators in the macroinvertebrates community was observed in the first months of the hydroperiods (except for hydroperiod 2), whereas a decreasing relative abundance of predators until the end of the hydroperiods was observed (except for Rajoleria in second and third hydroperiods). Anostraca were the most abundant for both first and third hydroperiods during the first three months, whereas Odonata dominated during the last month of the three hydroperiods. In the second hydroperiod, Diptera and

Figure 14. Relative abundance (%) of the main groups of zooplankton predators (PRED) and non-predators (NO PRED) macroinvertebrates in the 3 studied ponds along de hydroperiods (separated by thick black lines). Missing samples in Prats del Roser were due to the low water level during some months, which prevented the withdrawal of macroinvertebrates using the method previously specified.

Coleoptera dominated during the first month, whereas Odonata and Heteroptera dominated during the last month.

6.4.3 Size Spectra

A total of 81 size spectra were obtained (Figure 15-16, and Figures S1, S2, S3 in Supporting Information), and 90% of the spectra fit to a linear model ($R^2>0.7$, p-value <0.05). Six size spectra out of 81 were discarded. The average slope of size spectra of phytoplankton and zooplankton together (i.e., physiological scale) was -1.83 (±0.2), while the average slope of phytoplankton and zooplankton separately (i.e., ecological scale), was -1.4 (±0.4) and -1.2 (±0.2) respectively. As for the intercepts, the average of the physiological scale was 3.7 (±3.4), whereas for phytoplankton and zooplankton it was 10.7 (±5.1) and 3.4 (±2.6) respectively.



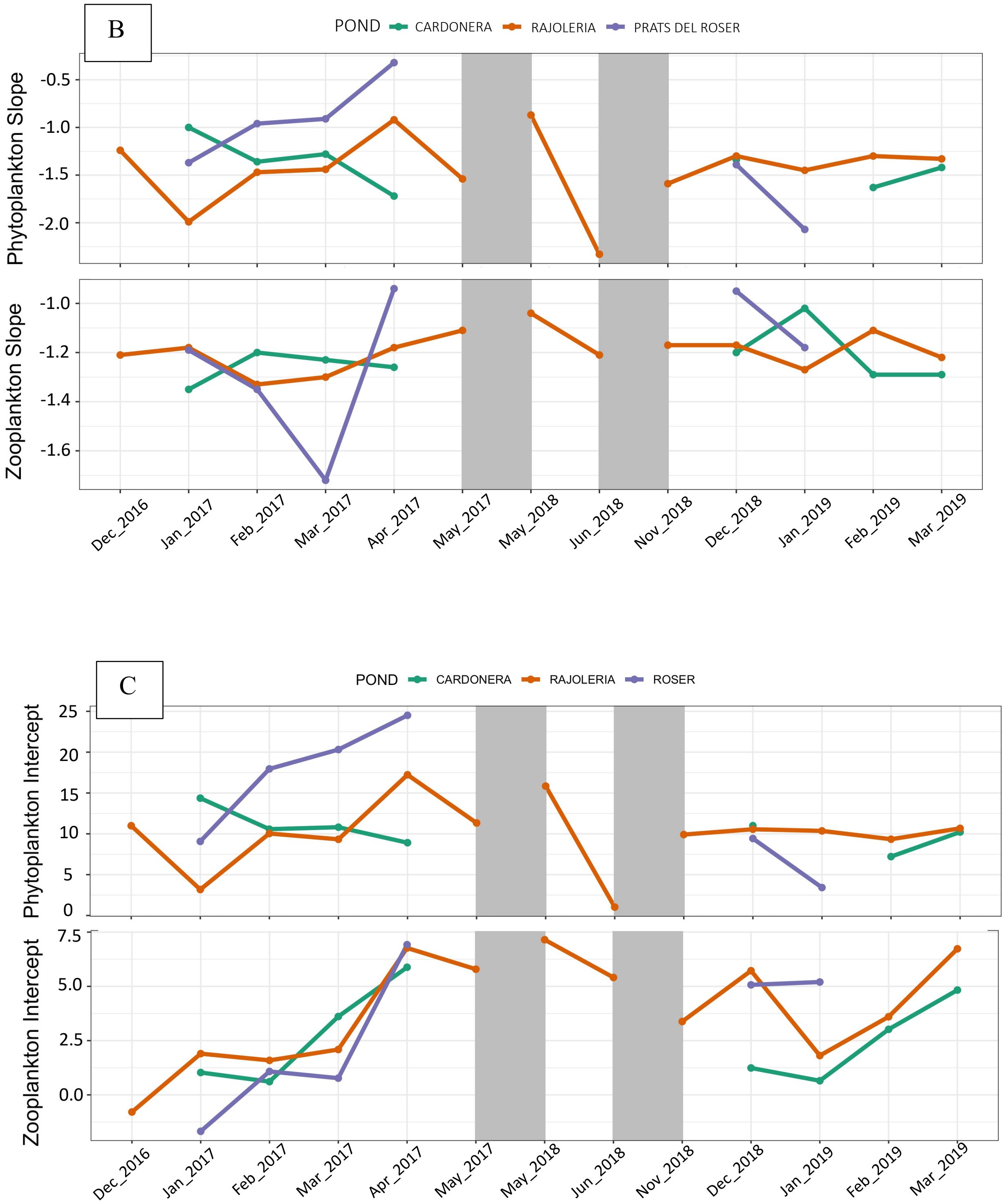


Figure 15. Temporal **e**volution of size spectrum intercept and slope along the three hydroperiods (separated by grey bars) in each of the three ponds studied: A) intercepts and slopes of phytoplankton and zooplankton together (physiological scaling), B) intercepts of phytoplankton and zooplankton (ecological scaling) and C) slopes of phytoplankton and zooplankton (ecological scaling)

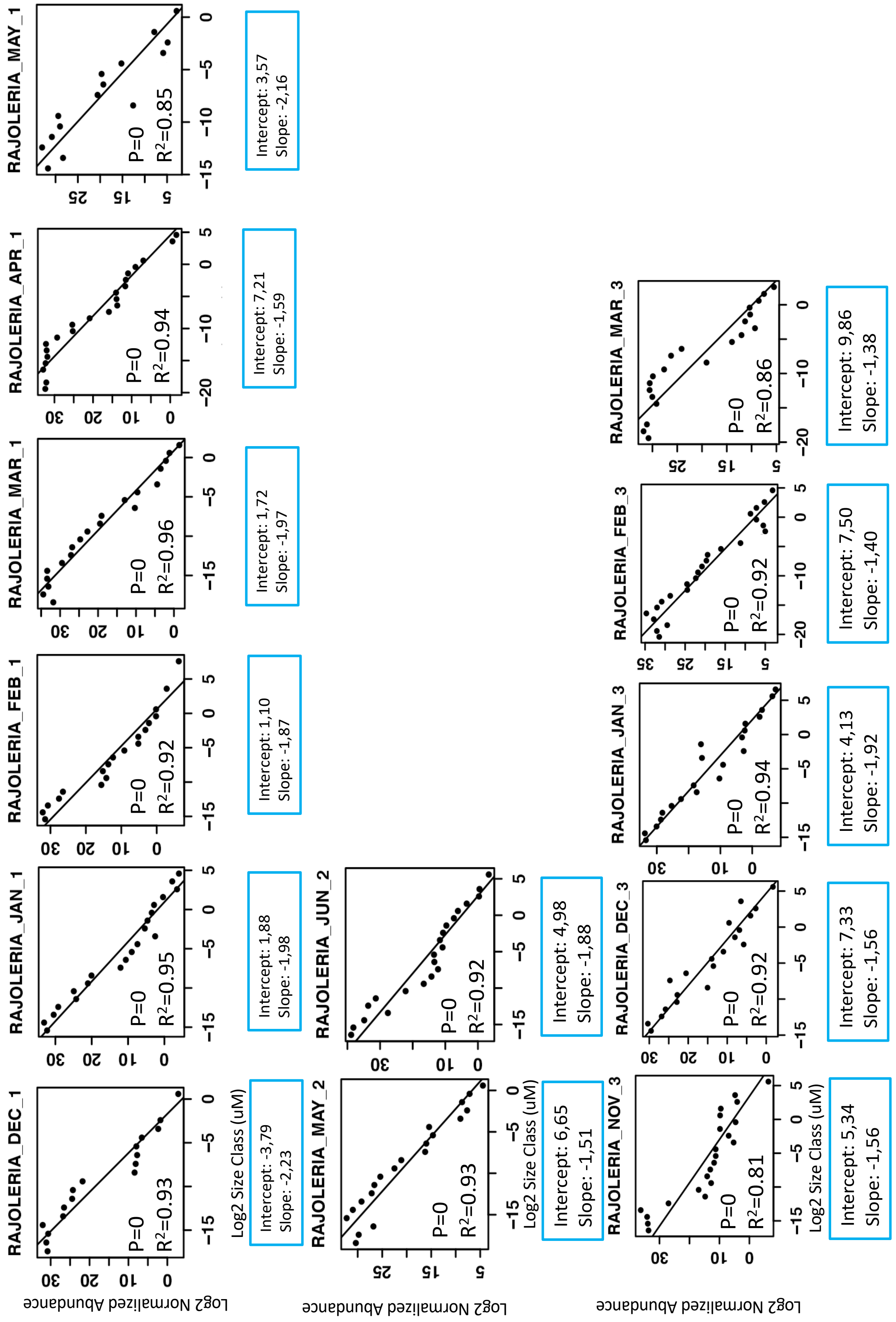

RAJOLERIA_DEC_1
P=0
R²=0.93
Intercept: -3,79
Slope: -2,23
RAJOLERIA_JAN_1
P=0
R²=0.95
Intercept: 1,88
Slope: -1,98
RAJOLERIA_FEB_1
P=0
R²=0.92
Intercept: 1,10
Slope: -1,87
RAJOLERIA_MAR_1
P=0
R²=0.96
Intercept: 1,72
Slope: -1,97
RAJOLERIA_APR_1
P=0
R²=0.94
Intercept: 7,21
Slope: -1,59
RAJOLERIA_MAY_1
P=0
R²=0.85
Intercept: 3,57
Slope: -2,16
RAJOLERIA_MAY_2
P=0
R²=0.93
Intercept: 6,65
Slope: -1,51
RAJOLERIA_JUN_2
P=0
R²=0.92
Intercept: 4,98
Slope: -1,88
RAJOLERIA_NOV_3
P=0
R²=0.81
Intercept: 5,34
Slope: -1,56
RAJOLERIA_DEC_3
P=0
R²=0.92
Intercept: 7,33
Slope: -1,56
RAJOLERIA_JAN_3
P=0
R²=0.94
Intercept: 4,13
Slope: -1,92
RAJOLERIA_FEB_3
P=0
R²=0.92
Intercept: 7,50
Slope: -1,40
RAJOLERIA_MAR_3
P=0
R²=0.86
Intercept: 9,86
Slope: -1,38
Log2 Normalized Abundance
Log2 Size Class (uM)

Figure 16. Physiological size spectra of phytoplankton and zooplankton together in Rajoleria, for each month sampled during the three hydroperiods (H1, H2, H3). On the bottom of each size spectrum, intercepts and slopes values are shown. The pond name is indicated on the top of each spectrum, together with the month. The number following the month indicates the hydroperiod. P-values and R^2 are reported inside each spectrum. P-values =0 mean that they are very significant (p-value <0.001).

6.4.4 Main Drivers of the Planktonic Size Spectrum slope and intercept

Results of Linear Mixed Models (Table 9) indicated that the most important drivers influencing size spectra parameters of phytoplankton and zooplankton were mean water column depth (DEPTH) and the PCA1 axis. Results showed a significant positive effect of the PCA1 axis, on both intercepts of phytoplankton and zooplankton size spectra, and a significant negative effect of mean water column depth on zooplankton intercept and on the whole planktonic intercept and slope. Beta coefficient of PCA1 axis and mean water column depth (0.29 and -0.41, respectively) in the zooplankton intercept model reflected a higher importance of mean water column depth on the abundance of zooplankton with respect to PCA1 axis. No significant effect of predation on size spectrum parameters were found (i.e., no relationship was found between zooplankton biomass and phytoplankton slope or intercept and neither between macroinvertebrate biomass and zooplankton slope nor intercept) (see the full models in Supplementary Table S4, section 10).

Table 9. Results of the Linear Mixed Models (N=25) showing the predictor variables that affect the slopes and the intercepts of the ecological (phytoplankton, zooplankton) and physiological (phytoplankton and zooplankton together) size spectra (SS). Only the most parsimonious significant models are presented. For each one, estimate, standard error (S.E.), Beta coefficients (standardized), *t*-value, significance (*p*-value) and degrees of freedom (df) are shown. For each model, R^2 (the proportion of variance expressed by predictors) are shown, together with the AIC (Akaike's Information Criterion). Significant predictor variables were PCA1 (scores for the PCA axis 1) and DEPTH (mean water column depth). Only the best models are shown.

Response variable	Predictor variable	Estimate	S.E.	Beta coefficients	*t* - Value	*p* - Value	df	R^2	AIC
Phytoplankton SS intercept	PCA1	0.49	0.19	0.5	2.65	0.02	19	0.24	68.66
Zooplankton SS intercept	DEPTH	-0.49	0.17	-0.41	-2.96	<0.01	18	0.4	62.61
	PCA1	0.39	0.16	0.29	2.5	0.02			
Phytoplankton + Zooplankton SS intercept	DEPTH	-2.15	0.5	-0.52	-4.31	<0.001	19	0.33	114.49
Phytoplankton + Zooplankton SS slope	DEPTH	-0.14	0.04	-0.39	-3.15	<0.01	19	0.23	2.82

For a graphical visualization of the results obtained, see Figure S6 in Supporting Information.

6.5 DISCUSSION

According to our study, changes in the environmental factors along the hydroperiod, rather than predation, are relevant in shaping the planktonic size spectrum of the studied Mediterranean temporary ponds. In general, our results suggest an increase in size spectrum intercepts at both ecological and physiological scaling (i.e., higher phytoplankton and zooplankton abundances) along the hydroperiod, thus when decreasing mean water column depth. In addition, an increase in the plankton resources (e.g., total nutrients and chlorophyll-*a*; PCA1 axis gradient) led to an increase in the ecological size spectrum intercepts (phytoplankton and zooplankton separately). Our results also showed a flattening of the planktonic size spectrum (at physiological scaling) as mean water column depth decreased throughout the hydroperiod.

In Mediterranean temporary ponds, environmental conditions change drastically showing higher temperatures, as hydroperiod progresses and ponds start drying (Bazzanti et al., 1996; Boix et al., 2004; Florencio et al., 2014). Such environmental changes were also observed in the studied ponds and were related with an increase in the size spectrum intercepts of the zooplankton community and the whole planktonic community (phytoplankton and zooplankton together), which suggests an increase in the organism's abundance due to both population growth and organisms' concentration along the hydroperiod. Phytoplankton and zooplankton size spectrum intercepts were also influenced by nutrient concentration (TN and TP) and chlorophyll-*a* (i.e., PCA1 axis), which suggests that their abundances also increased with resource availability, irrespectively of the mean water column depth. In fact, although a general trend of increasing PCA1 axis along the hydroperiod was observed in this study, no correlation was found between mean water column depth and nutrient concentration or chlorophyll-*a* axis. This was probably because nutrients' concentration in the ponds also increased during rainfall and runoff events and not only due to a concentration effect when ponds were drying out.

According to theoretical expectations (Polishchuk & Blanchard, 2019), the slopes of the planktonic size spectra (i.e., phytoplankton and zooplankton together; physiological scaling) should be -2 (for the normalized abundance size spectra, as used in this study). In the studied ponds, the slopes of the physiological size spectrum were close to the theoretical value, but our results also showed that the slope systematically changed with mean water column depth, with flatter slopes with decreasing water column (i.e., higher relative abundance of larger individuals in the planktonic community (e.g., cladocerans or large copepods)). Since flatter slopes reflect an increase in the trophic transfer efficiency along the food web (Brown et al., 2004; Mehner et al., 2018; Treblico et al., 2013), our results suggest an improvement in the energy transfer as water hydroperiod progresses. Nevertheless, the benthic production likely plays an additional role in the increase of the relative abundances of large size individuals in the planktonic food web when water level is low, thus, future studies should take this into account to have better mechanistic understanding of the patterns of energy transfer in the food web of temporary ponds. A similar pattern of flattening of the slope of community size spectrum (zooplankton and macroinvertebrates) along the hydroperiod was observed in Mediterranean ponds and was explained by both the sequential development of the community from smaller to larger individuals and the macroinvertebrates predation on zooplankton (Boix et al., 2004). Similarly, in Mediterranean brackish temporary ponds a flattening of the slope of zooplankton size spectra was observed according to the hydrological cycle (Brucet, Boix, et al., 2005), due to the entry of nutrients and thus, food availability for zooplankton. Although flatter slopes have been related with increasing resource availability (Ahrens & Peters, 1991; Gaedke et al., 2004; Sprules & Munawar, 1986; Zhang et al., 2013), this was not reflected in our study where planktonic size spectrum slopes were only related to changes in mean water column depth.

Regarding the effects of predation on the planktonic size spectrum parameters, and contrary to our expectations, no relationships were found between predators and the size spectrum parameters of preys (i.e., neither for zooplankton grazing on phytoplankton nor for the macroinvertebrate predation on zooplankton). A study in a Mediterranean temporary pond (Boix et al., 2004) showed a rise in trophic interactions such as predation and competition during the last phase of the hydroperiod due to the higher abundances of macroinvertebrates, whose predation on zooplankton was partially responsible for the flattering of size spectrum slope (decreasing zooplankton relative abundances while increasing macroinvertebrates). Similar dynamics were observed in non-Mediterranean temporary ponds (Lahr et al., 1999; Lake et al., 1989). In our study, abundances of planktivorous macroinvertebrates were low (mean of 28.1, 29.5, 16.6 individuals per sample in the hydroperiod 1, hydroperiod 2 and hydroperiod 3 respectively, considering the three ponds together), and tended to decrease along the hydroperiod. Although the hydroperiods length in our ponds (from 60 to 180 days) were long enough to allow the completion of the long-life cycles of the larger predators (Barclay, 1966; Kenk, 1949; Schneider, 1999), abundance of coleopterans was quite low (mean of 6 individuals/sample), and it decreased along the hydroperiod, whereas odonates only appeared, in low abundance (mean of 2.5 individuals/sample), during the last month of each hydroperiod. One of the reasons for this low abundance could be the presence of amphibians such as *Pelophylax perezi*, *Triturus marmoratus*, *Hyla meridionalis*, *Pelobates cultripes* and *Epidalea calamita* (unpublished data), whose main diet is composed by macroinvertebrates, many of which also feed on zooplankton (i.e., Coleopterans, Heteropterans, Odonates among others; Burghelea et al., 2010). Similarly, this absence of predation effects on size spectrum slope has also been observed in a recent study where slope was relatively robust against changes in predation strength over successive years (Braun et al., 2021). These authors explained the absence of fish predation effects on zooplankton size spectrum because predators preferred to prey on some zooplankton species which were of small size and in small abundances, causing no modification of the zooplankton size spectrum. Thus, we can neither discard that macroinvertebrate predation in our study is more species-selective rather than size-selective. A size-based predation of zooplankton on phytoplankton was neither observed in the present study. This could be due to the high abundances of phytoplankton colonies present in the samples. Colonial forms are non-edible for zooplankton since they clog the feeding apparatus of the zooplankton, reducing their grazing rates (Ersoy et al., 2017; Paerl & Otten, 2013; Webster & Peters, 1978).

In conclusion, in the studied Mediterranean temporary ponds the changes in the environmental variables along the hydroperiod were reflected in the shape of the size spectrum of planktonic community. The increase in resource availability-related variables such as total nutrients and chlorophyll-*a* (PCA1 axis gradient) led to an increase in phyto- and zooplankton abundances (i.e., size spectrum intercept). The decrease in mean water column depth was also related to an increase in the size spectrum intercepts of zooplankton, and phytoplankton and zooplankton together, which suggests an increase in the organism's abundance, because of both population growth and organisms' concentration along the hydroperiod. A flattening of planktonic size spectra slope was also observed along the hydroperiod, showing the increase in the relative abundance of larger zooplankton individuals. Regarding predation effects, size spectrum parameters (both at ecological and physiological scaling) were robust against changes in predation pressure along the hydroperiod. All together our results suggests that the size structure of plankton communities is sensitive to the environmental changes and reflects the communities' dynamics that occur along the periodic cycles of flooding and drought in temporary Mediterranean ponds.

7. GENERAL DISCUSSION

The present thesis aimed to discern the main factors which are responsible for changes in the size structure of the different trophic levels of the food web in Mediterranean ponds. The results suggest that environmental factors such as nutrient availability, conductivity, mean water column depth, and water quality are the main responsible in shaping the size structure of the aquatic communities studied (phytoplankton and zooplankton communities for Chapters 1 and 3, and fish populations for Chapter 2). Although trophic interactions are known to be size dependent, in the present thesis biotic interactions were only marginally related to changes in the size structure of the studied communities (i.e., phytoplankton size diversity in chapter 1). Despite the different environments considered (brackish, freshwater, temporary and permanent), some common variables were found to shape communities' size structure in Mediterranean ponds, as discussed below.

Size structure of aquatic communities in Mediterranean ponds: considerations and implications

The size structure of the aquatic communities, including plankton and fish populations, was analyzed considering different size metrics: geometric mean size, maximum size, size range, size diversity and normalized abundance size spectrum. The use of size metrics such as mean, maximum and body size range is a simple but valuable approach commonly used in many aquatic and terrestrial organisms since these metrics respond to variations in both biotic and abiotic gradients (Gardner et al., 2014; Tryjanowski et al., 2006; Woodward & Hildrew, 2002), as in the second chapter of the present thesis, where mean and maximum size and size range of *A. iberus* responded to environmental changes in the ponds. Recently these variables have been employed to evaluate the effects of anthropogenic modifications in aquatic ecosystems (i.e., tourism and agricultural land use) and the global warming on organisms' size structure (i.e., decreasing body size with increasing temperature) (Arranz, et al., 2016; Benejam et al., 2018; Brucet et al., 2010; Emmrich et al., 2014; Gardner et al., 2011). Nevertheless, these size metrics are not as explicative as other size-based indicators (e.g., size diversity, size spectrum), as they do not integrate the variability in the body size distributions.

The size spectrum (i.e., negative linear relationship between individual body size and abundance at logarithmic scale) is a useful tool to summarize ecosystem information (Kerr & Dickie, 2001) such as trophic interactions (Boix et al., 2004; Brucet, Boix, et al., 2005; Quintana et al., 2006; Zimmer et al., 2001), environmental conditions (Ahrens & Peters, 1991; Sprules & Munawar, 1986; Zhang et al., 2013) and also the Trophic Transfer Efficiency (TTE) in the food web (Mehner et al., 2018). Nevertheless, it is difficult to obtain conclusions about the trophic transfer efficiency in the planktonic food web of shallow water bodies, like the ones studied in this thesis, since a benthos and plankton coupling appears when water level is low, making it difficult to distinguish between the two environments. On the other hand, size spectrum seems to be quite useful when dealing with temporal dynamics since changes in the community or the food web (e.g., along the hydroperiod, Chapter 3) are directly translated into changes in the shape of the size spectra (i.e., variability in the size spectrum slope and intercept). As performed in the present thesis (Chapter 3), different scaling can be used to model the size spectra: the ecological scaling, considering a single functional group/trophic level (e.g., phytoplankton or zooplankton; (Dickie, 1987)) and physiological scaling, considering several groups together (e.g., phytoplankton + zooplankton; (Sprules & Barth, 2016)). Both approaches are known to give complementary information on the food web functioning (Dickie, 1987). For instance, in the temporary ponds the resource availability-related variables (e.g., total nutrients, chlorophyll-*a*, etc..) were found to be related only to the phytoplankton and zooplankton size spectra (ecological scaling) whereas the physiological spectra, including both groups together, were related to other abiotic variables (Chapter 3). Nevertheless, on some occasions size spectrum cannot be modelled because of the low number of individuals (e.g., macroinvertebrates in Chapter 3) or because it does not properly adjust to linear or non-linear models (e.g., phytoplankton and zooplankton in Chapter 1, data non shown). In these situations, other size metrics such as the size diversity index can be a proper alternative to study the community size structure.

The size diversity index, which is analogous to Shannon-Wiener species diversity index but adapted for continuous variables such as body size, condenses in a single value per size distribution different aspects of other size metrics (Brucet et al., 2006; Emmrich et al., 2011; Quintana et al., 2008). The value of the size diversity index is comparable among size distributions within and between communities, as well as among ecosystems, as are the species diversity indices, and it is easy to interpret since the concept of diversity has been well established (Quintana et al., 2008). It has the advantage to avoid the arbitrariness introduced when using size classes to calculate the size spectrum and it systematically responds to environmental variation. Moreover, it has been shown that size diversity index can be complementary to the Shannon-Wiener diversity index when studying structure of aquatic communities since the former being more affected by size-based interactions (e.g., competition and predation) whereas the latter being more determined by abiotic factors (Badosa et al., 2007; Gascón, Boix, Sala, et al., 2009). Partially supporting this idea, results from the first chapter showed clearly that planktonic species diversity (i.e., Shannon diversity of phytoplankton and zooplankton) responded only to differences in pond surface (i.e., area), whereas size structure of the planktonic community (phytoplankton and zooplankton), responded to biotic and abiotic variables, such as nutrient's availability (which may induce competition among individual sizes) and conductivity.

Drivers shaping the size structure of aquatic communities in Mediterranean ponds

The present thesis shows that size metrics can provide valuable information at different ecological scales: at functional group level (such as phytoplankton and zooplankton in Chapter 1), at a population level (i.e., *Aphanius iberus*, Chapter 2), and integrating different trophic levels (phytoplankton + zooplankton in Chapter 3), as each organization level can respond in a different way to environmental and biotic factors. Also, the combination of different size metrics is a good way to ensure that the response of organisms' size structure along environmental gradients is adequately captured.

Environmental factors such as resource availability (e.g., nutrient concentrations), water quality, conductivity and water column depth were more important than biotic factors (i.e., predation) in determining the size structure of planktonic communities and *A. iberus* populations in the studied Mediterranean ponds. Thus, an increase in resource-related variables (e.g., chlorophyll-*a,* total nitrogen and phosphorus) was related with an increase of the phytoplankton and zooplankton size spectrum intercepts (i.e., higher abundances) in temporary ponds due to population growth (Chapter 3), as an increase in availability of nutrients creates more resources for the communities, which is translated to an increase in abundance of individuals in all the size classes (Ahrens & Peters, 1991; Boudreau & Dickie, 1992; Sprules & Munawar, 1986). In *Aphanius iberus* populations, higher values of the different size metrics studied (e.g., maximum and mean size, size range and size diversity), as well higher abundances, were also found under high concentrations of total nitrogen, which are characteristic of the more confined brackish ponds (Quintana, 2002). More than a direct effect of total nitrogen on *A. iberus* populations, this was probably related to the fact that in more confined ponds population' size of *A. iberus* was larger due to the lack of external perturbations such as the presence of invasive competitor species (which are less salt-tolerant) and/or the freshwater inputs (Prado et al., 2017). It is worth to mention that size structure of *A. iberus* was also affected by the water quality of the ponds (evaluated by using the *QAELS* index for lentic and shallow ecosystems; (Boix et al., 2005)) since a better quality seem to favor the presence of bigger fish sizes (i.e., higher maximum and mean fish size), highlighting the importance of pond ecological status for the conservation of this endemic and endangered fish species.

The effect of increasing salinity on fish in wetlands have been widely discussed in bibliography (Bilkovic et al., 2012; Gelwick et al., 2001; Hart et al., 1990; Lorenz & Serafy, 2006) among many others, but the effects of salinity on the euryhaline cyprinodont *Aphanius iberus*' size structure are much less considered. In the present study, higher salinities had a negative effect on the mean and maximum individual size of *A. iberus* populations in brackish ponds (Chapter 2). *A. iberus* is known to tolerate wide ranges of temperature and salinity variations (Oltra & Todolí, 2000; Vargas & De Sostoa, 1997) allowing its survival under high salinity levels even though its metabolism may be affected by such high salinities (Brett & Groves, 1979; Oliva-Paterna et al., 2006; Plaut, 2000; Wootton, 1989; Yildirim & Karacuha, 2008). This could explain the observed decrease of *A. iberus'* mean and maximum size in the brackish ponds where salinity was higher, because the energy used for osmoregulation is not available for their growth performance (Brett & Groves, 1979; Wootton, 1989; Yildirim & Karacuha, 2008). Nevertheless, high salinity ponds are known to act as a "refuge" for *A. iberus* since it reduces the colonization success of one of its main competitors, the less salt-tolerant fish species *G. holbrooki* (Leonardos & Sinis, 1998).

Water column depth was another important driver of the size structure of planktonic communities and *A. iberus* populations. In deeper brackish ponds, a large range of individual sizes, as well as higher abundances, was found for *A. iberus* populations, as different developmental stages could occupy different habitats throughout the water column. Thus, in deeper water bodies there is a higher probability to find a wider range of fish sizes (Arranz et al., 2016; Søndergaard et al., 2005). In temporary ponds, the size spectrum of the planktonic community, at both ecological and physiological scaling, responded to temporal changes in the water column depth since higher abundances (i.e., higher intercepts) and an increase in the relative abundance of larger organisms (i.e., flatter slope) were found at the end of hydroperiod. A similar pattern was observed in other Mediterranean temporary ponds, considering physiological spectra of aquatic invertebrates (Boix et al., 2004) and ecological size spectra of zooplankton (Brucet, Boix, et al., 2005).

In the present study, and contrary to our expectations, biotic interactions (i.e., predation) did not affect the size structure of planktonic communities in Mediterranean ponds. Despite expecting an effect of the size-based predation among the different trophic levels (Boix et al., 2004; Ye et al., 2013), no predation effect has been observed in the present thesis, neither zooplankton grazing on phytoplankton nor fish or macroinvertebrates predation on zooplankton. Thus, some considerations have to be made before making categorical statements on the results found.

Fish predation is the most important driver of zooplankton body size structure in many ecosystems, and previous studies found significant relationships between predators and preys size diversity (Brucet et al., 2017; Mehner et al., 2016). Nevertheless, the similar body size ranges of the fish community in the studied brackish ponds (*A. iberus*, *G. holbrooki* and *A. boyeri*), may explain that no size-based predation effect on zooplankton was observed. In addition, fish predation could have been masked by the presence of other top-predators such as the jellyfish *Odessia maeotica* and the amphipod *Gammarus aequicauda* (Compte et al., 2010, 2011), observed *in-situ* in some of the studied ponds during the samplings.

The low abundance of macroinvertebrates predators (i.e., Odonates, Coleopterans etc..) in temporary ponds during the sampling period may have prevented to observe significant predation effects on the planktonic size spectrum. Although the hydroperiods' lengths were long enough to allow the completion of the long-life cycles of the larger predators (Barclay, 1966; Kenk, 1949; Schneider, 1999), other factors could be responsible for this low abundance. One of them could be the presence of amphibians such as *Pelophylax perezi*, *Triturus marmoratus*, *Hyla meridionalis*, *Pelobates cultripes* and *Epidalea calamita* (unpublished data), whose main diet is composed by macroinvertebrates, many of them planktivorous (i.e., Coleopterans, Heteropterans, Odonates among others; (Burghelea et al., 2010). Nevertheless, this interaction was not taken into account in the present thesis. It cannot be discarded that macroinvertebrate predation was more species-selective rather than size-selective, as it has been observed for fish predation on zooplankton in previous studies (Braun et al., 2021), preventing to observe any size-based predation effect on zooplankton.

Although some studies found positive (Fox, 2004) and negative (García-Comas et al., 2016) relationships between zooplankton and phytoplankton size diversity, in the present thesis zooplankton grazing did not significantly modify phytoplankton' size diversity. This thesis showed that under food scarcity (i.e., higher ratio phytoplankton: total nitrogen), few size classes are favoured, especially the smallest ones, which are known to be more efficient feeders than the large ones (Guidi et al., 2009; Litchman & Klausmeier, 2008). In addition, the high presence of non-edible phytoplankton (i.e., colonial species) disfavored its assimilation by zooplankton, as observed in previous studies (DeMott et al., 2001; Ersoy et al., 2017).

It is necessary to be aware that working with different temporal scales has also different implications when interpreting the results obtained. In fact, whereas the 13 brackish ponds were sampled once during the same period (i.e., late spring-summer of 2016), the 3 temporary ponds were sampled monthly along the hydroperiod, for three consecutive years. The main consideration that needs to be addressed is that the first sampling was a 'snapshot' of a particular situation in a concrete moment, being a mere comparison among ponds with different dynamics and species compositions. Thus, it may miss temporal implications, such as differences in population or community dynamics depending on the season, which prevent to generalize the results. On the other hand, the temporal monitoring of a pond, as in chapter 3, can bring insights into how the studied communities evolve and change in time along with the modifications derived from the hydroperiod progressing. Thus, temporal data give a more complete picture of the dynamics, allowing to observe modification of the same populations or community across time, but, in the case of chapter 3, without considering a wide spatial variability. Both approaches would be necessary to obtain a comprehensive understanding of the system functioning.

8. FUTURE WORK AND CONSIDERATIONS

The current thesis is, to my knowledge, one of the few works on the size structure of planktonic communities and populations of Mediterranean ponds including three trophic levels (phytoplankton, zooplankton, and fish). Nevertheless, as in many ecological studies performed in a natural environment, results obtained in the present work have been inevitably limited to some restrictions, such in the case of the number of ponds studied in the Chapter 1 and 2 (N=13). which may limit ultimately the statistical reliability. Nevertheless, in the second chapter working with *A. iberus* populations, this point was improved by considering the fyke net as a single observation previous correction for pseudo replication. Sampling season (late spring) in Chapter 1 and 2 could be seen as another limitation for conclusions obtained on brackish ponds. Although it was the optimal period to observe a broader peak of plankton and the different developmental stages of *Aphanius iberus*, results were circumscribed to a determined period of the year. Sampling during different seasons should be considered in the future to rule out the effect of seasonality. In addition, the present study encompasses only a portion of the endemic cyprinodont's natural distribution, being restricted to "Aiguamolls de l'Empordà" and "El Montgrí, Illes Medes i el Baix Ter" Natural Parks. Further studies on *A. iberus* should include a wider range of its distribution, in order to increase the knowledge on the factors which most affect the size structure of this species, which is very important to be aware of the populations' status and to guarantee the conservation of the species.

It is necessary to be aware that working with different temporal scales has also different implications when interpreting the results obtained. In fact, whereas the 13 brackish ponds were sampled once during late spring (Chapters 1 and 2) the 3 temporary ponds (Chapter 3) were sampled monthly along the hydroperiod, for three consecutive years. The main consideration that needs to be addressed is that spring sampling was a 'snapshot' of a particular situation allowing to perform only a comparison among ponds in a specific moment. The fact of not including seasonal variation in population or community dynamics prevent to generalize the results. In Chapter 3, although pond studied did not cover a wide spatial variability, the temporal monitoring of three freshwater ponds through three consecutive hydroperiods brought insights into how the studied communities evolved and changed in time along with the modifications derived from the hydroperiod progress. Both approaches would be necessary to obtain a comprehensive understanding of the system functioning.

The thesis has shown that the slope of the size spectrum (i.e., proxy of the trophic transfer efficiency) of the planktonic food web responds to changes in the environmental conditions in temporary ponds. However, conclusions on trophic transfer efficiency are limited, as benthos energy transfer was not taken into account. When studying shallow wetlands, it is difficult to distinguish between planktonic and benthic organisms, as water level can have high fluctuations and organisms may move between the two habitats, so the distinction is not clear. Future studies should aim at integrating the planktonic and benthic size spectrum in order to get a better scenario of the changes in the trophic transfer efficiency in the food web of temporary ponds. For this purpose, an even more complete analysis of the trophic interactions should be considered, including the potential effects of omnivory, the different sources of food of the trophic levels considered, intraguild predation, and the presence of the microbial loop.

9. CONCLUSIONS

- Phytoplankton size diversity in brackish ponds during spring season was affected by nutrient limitation (i.e., bottom-up effects) rather than by zooplankton predation (i.e., top-down effects). Low phytoplankton size diversity, together with a dominance of small cells, was found when resource availability was low. This was likely due to the fact that small phytoplankton is more efficient under food scarcity due to their low resource requirements and higher surface:volume ratio.

- Zooplankton size diversity in brackish ponds during spring season was not affected by any of abiotic and biotic factors (i.e., fish predation) tested. Similar fish size diversity among ponds likely prevented a significant effect of fish predation on zooplankton.

- Taxonomic diversity of both phytoplankton and zooplankton in brackish ponds during spring season was only affected by abiotic factors (i.e., pond morphometry variables). Thus, higher taxonomic diversities were found in a larger pond maybe because higher habitat heterogeneity likely favors the coexistence of more species.

- The size structure of *Aphanius iberus* populations in brackish ponds during spring season was mainly shaped by the following environmental variables: water column depth, total nitrogen concentration and water quality (*QAELS* index), which were positively related with some of the size metrics considered (e.g., mean and maximum individual size, individual size range and size diversity). In constract, conductivity and zooplankton biomass (as food resource) were negatively related with mean and maximum length. Water column depth was also positively related with *A. iberus* abundance.

- Range of individual length and abundance (i.e., CPUE) of *A. iberus* were found to increase with water column depth, as wider and deeper waterbodies are known to host greater biomass and size of fish, with the consequent higher probability to find a wider range of fish sizes. A higher range of individual length of the fish, together with its maximum body length, and size diversity, were also related with high nitrogen concentrations. More than a direct effect of total nitrogen, these results suggested that in the more confined brackish ponds (where total nutrients and organic matter tends to accumulate over years), the populations of *A. iberus* are more protected from external perturbations such as freshwater inputs and the presence of freshwater invasive species.

- Water quality of the ponds (*QAELS* index) was also a determinant for the size structure of *A. iberus* populations since larger maximum and mean individual lengths were found at higher water quality conditions.

- The size structure of *A. iberus* populations is negatively affected by water conductivity since maximum and mean individual lengths were lower under high conductivities. Although high salinities prevent the presence in the ponds of potential competitors that are less salt-tolerant (e.g., *Gambusia holbrooki*), they may hinder *A. iberus* osmoregulatory abilities, affecting the growth performance and leading to smaller-sized fish. The same parameters (maximum and mean individual lengths) of A. *iberus* were negatively related with zooplankton biomass (i.e., food resource). This result could be explained by the fact that consumption rates in fish increase with body size.

- Phytoplankton and zooplankton abundances (i.e., size spectrum intercept at ecological scaling) in temporary ponds where positively related with resource-related variables (e.g., chlorophyll-*a*, total nitrogen, and phosphorus). An increase in the resource availability was translated to an increase in abundance of individuals in all the size classes due to populations growth.

- Physiological size spectrum (phytoplankton + zooplankton) parameters and the size spectrum intercept of zooplankton were negatively related to water column depth. The decrease in water column depth along the hydroperiod led to an increase in the organisms' abundance (i.e., high intercepts) probably related to a concentration effect when ponds were drying out. The flattening of the size spectrum slope along the hydroperiod was explained by an increase in the relative abundances of bigger zooplankton, suggesting an improvement in the energy transfer along the food web as hydroperiod progresses.

- Although phytoplankton and zooplankton body size are known to be mainly affected by predation, in the studied temporary ponds no predation effects on planktonic community size structure were observed, neither zooplankton grazing on phytoplankton nor macroinvertebrates predation on zooplankton. These results could be due, respectively, to the high presence of phytoplankton colonies, (i.e., non-edible for zooplankton) and the low abundances of the main macroinvertebrates predators (e.g., Odonates, Coleopterans).

10. REFERENCES

Ahrens, M. A., & Peters, R. H. (1991). Patterns and limitations in limnoplankton size spectra. *Canadian Journal of Fisheries and Aquatic Sciences*, *48*(10), 1967–1978. https://doi.org/10.1139/f91-234

Alcaraz, C., Bisazza, A., & García-Berthou, E. (2008). Salinity mediates the competitive interactions between invasive mosquitofish and an endangered fish. *Oecologia*, *155*(1), 205–213. https://doi.org/10.1007/s00442-007-0899-4

Alcaraz, C., & García-Berthou, E. (2007). Life history variation of invasive mosquitofish (Gambusia holbrooki) along a salinity gradient. *Biological Conservation*, 139(1-2), 83-92. https://doi.org/10.1016/j.biocon.2007.06.006

Alcaraz, C., Pou-Rovira, Q., & García-Berthou, E. (2008). Use of a flooded salt marsh habitat by an endangered cyprinodontid fish (*Aphanius iberus*). *Hydrobiologia*, 600(1), 177-185. https://doi.org/10.1007/s10750-007-9230-y

Almond, M. J. R., Bentzen, E., & Taylor, W. D. (1996). Size structure and species composition of plankton communities in deep Ontario lakes with and without Mysis relicta and planktivorous fish. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*(2), 315–325. https://doi.org/10.1139/f95-184

Alvarez Cobelas, M., Rojo, C., & Angeler, D. G. (2005). Mediterranean limnology: Current status, gaps and the future. *Journal of Limnology*, *64*(1), 13–29. https://doi.org/10.4081/jlimnol.2005.13

Anton-Pardo, M., & Armengol, X. (2012). Effects of salinity and water temporality on zooplankton community in coastal Mediterranean ponds. *Estuarine, Coastal and Shelf Science*, *114*, 93–99. https://doi.org/10.1016/j.ecss.2011.08.018

Arranz, I., Mehner, T., Benejam, L., Argillier, C., Holmgren, K., Jeppesen, E., Lauridsen, T. L., Volta, P., Winfield, I. J., Brucet, S., & Shuter, B. (2016). Density-dependent effects as key drivers of intraspecific size structure of six abundant fish species in lakes across Europe. *Canadian Journal of Fisheries and Aquatic Sciences*, *73*(4), 519–534. https://doi.org/10.1139/cjfas-2014-0508

Atkinson, D., Ciotti, B. J., & Montagnes, D. J. S. (2003). Protists decrease in size linearly with temperature: ca. 2.5%°C -1. *Proceedings of the Royal Society B: Biological Sciences*, *270*(1533), 2605–2611. https://doi.org/10.1098/rspb.2003.2538

Àvila, N., López-Flores, R., & Quintana, X. D. (2018). Composition of pelagic microbial communities in Mediterranean coastal aquatic ecosystems under extreme drought conditions. *Estuarine, Coastal and Shelf Science*, 216, 139-147. https://doi.org/10.1016/j.ecss.2018.01.018

Badosa, A., Boix, D., Brucet, S., López-Flores, R., Gascón, S., & Quintana, X. D. (2007). Zooplankton taxonomic and size diversity in Mediterranean coastal lagoons (NE Iberian Peninsula): Influence of hydrology, nutrient composition, food resource availability and predation. *Estuarine, Coastal and Shelf Science*, 71(1-2), 335-346.https://doi.org/10.1016/j.ecss.2006.08.005

Badosa, A., Boix, D., Brucet, S., López-Flores, R., & Quintana, X. D. (2006). Nutrients and zooplankton composition and dynamics in relation to the hydrological pattern in a confined Mediterranean salt marsh (NE Iberian Peninsula). *Estuarine, Coastal and Shelf Science*, *66*(3–4), 513–522. https://doi.org/10.1016/j.ecss.2005.10.006

Bagella, S., Gascón, S., Filigheddu, R., Cogoni, A., & Boix, D. (2016). Mediterranean Temporary Ponds: new challenges from a neglected habitat. *Hydrobiologia*, *782*(1), 1–10. https://doi.org/10.1007/s10750-016-2962-9

Bagella, S., Satta, A., Floris, I., Caria, M. C., Rossetti, I., & Podani, J. (2013). Effects of plant community composition and flowering phenology on honeybee foraging in Mediterranean sylvo-pastoral systems. *Applied Vegetation Science*, *16*(4), 689–697. https://doi.org/10.1111/avsc.12023

Barbour, C. D., & Brown, J. H. (1974). Fish Species Diversity in Lakes. *The American Naturalist*, 108 (962), 473-489. https://doi.org/10.1086/282927

Barclay, M. H. (1966). An ecological study of a temporary pond near Auckland, New Zealand. *Marine and Freshwater Research*, *17*(2), 239–258. https://doi.org/10.1071/MF9660239

Barone, R., Castelli, G., & Naselli-Flores, L. (2010). Red sky at night cyanobacteria delight: The role of climate in structuring phytoplankton assemblage in a shallow, Mediterranean lake (Biviere di Gela, southeastern Sicily). *Hydrobiologia*, *639*(1), 43–53. https://doi.org/10.1007/s10750-009-0016-2

Bazzanti, M., Baldoni, S., & Seminara, M. (1996). Invertebrate macrofauna of a temporary pond in Central Italy: composition, community parameters and temporal succession. *Archiv Für Hydrobiologie*, 77–94.

Beja, P., & Alcazar, R. (2003). Conservation of Mediterranean temporary ponds under agricultural intensification: An evaluation using amphibians. *Biological Conservation*, *114*(3), 317–326. https://doi.org/10.1016/S0006-3207(03)00051-X

Beklioglu, M., Ince, O., & Tuzun, I. (2003). Restoration of the eutrophic Lake Eymir, Turkey, by biomanipulation after a major external nutrient control I. *Hydrobiologia*, *490*, 93–105. https://doi.org/10.1023/A:1023466629489

Benejam, L., Tobes, I., Brucet, S., & Miranda, R. (2018). Size spectra and other size-related variables of river fish communities: systematic changes along the altitudinal gradient on pristine Andean streams. *Ecological Indicators*, *90*(December 2017), 366–378. https://doi.org/10.1016/j.ecolind.2018.03.012

Benke, A. C., Huryn, A. D., Smock, L. A., & Wallace, J. B. (1999). Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society*, *18*(3), 308–343. https://doi.org/10.2307/1468447

Biggs, J., von Fumetti, S., & Kelly-Quinn, M. (2017). The importance of small waterbodies for biodiversity and ecosystem services: implications for policy makers. *Hydrobiologia*, *793*(1), 3–39. https://doi.org/10.1007/s10750-016-3007-0

Bilkovic, D. M., Mitchell, M. R., Hershner, C. H., & Havens, K. J. (2012). Transitional wetland faunal community characterization and response to precipitation-driven salinity fluctuations. *Wetlands*, *32*(3), 425–437. https://doi.org/10.1007/s13157-012-0276-x

Blanco, S., Romo, S., Villena, M. J., & Martínez, S. (2003). Fish communities and food web interactions in some shallow Mediterranean lakes. *Hydrobiologia*.506(1), 473-480. https://doi.org/10.1023/B:HYDR.0000008583.05327.da

Blaustein, L., & Schwartz, S. S. (2001). Why study ecology in temporary pools? *Israel Journal of Zoology*, *47*(4), 303–312. https://doi.org/10.1092/CKMU-Q2PM-HTGC-P9C8

Blondel & Aronson. (1999). Biology and Wildlife of the Mediterranean Region. In *Oxford Press*. https://doi.org/10.1016/s0006-3207(00)00013-6

Blumenshine, S. C., Lodge, D. M., & Hodgson, J. R. (2000). Gradient of fish predation alters body size distributions of lake Benthos. *Ecology*, *81*(2), 374–386. https://doi.org/10.1890/0012-9658(2000)081[0374:gofpab]2.0.co;2

Boix, D., Biggs, J., Céréghino, R., Hull, A. P., Kalettka, T., & Oertli, B. (2012). Pond research and management in Europe: "Small is Beautiful." *Hydrobiologia*, *689*(1), 1–9. https://doi.org/10.1007/s10750-012-1015-2

Boix, D., Gascón, S., Sala, J., Badosa, A., Brucet, S., López-Flores, R., Martinoy, M., Gifre, J., & Quintana, X. D. (2008). Patterns of composition and species richness of crustaceans and aquatic insects along environmental gradients in Mediterranean water bodies. *Hydrobiologia*, *597*(1), 53–69. https://doi.org/10.1007/s10750-007-9221-z

Boix, D., Gascón, S., Sala, J., Martinoy, M., Gifre, J., & Quintana, X. D. (2005). A new index of water quality assessment in Mediterranean wetlands based on crustacean and insect assemblages: The case of Catalunya (NE Iberian peninsula). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 15(6) 635-651. https://doi.org/10.1002/aqc.750

Boix, D., Sala, J., & Moreno-Amich, R. (2001). The faunal composition of espolla pond (NE Iberian Peninsula): The neglected biodiversity of temporary waters. *Wetlands*, *21*(4), 577–592. https://doi.org/10.1672/0277-5212(2001)021[0577:TFCOEP]2.0.CO;2

Boix, D., Sala, J., Quintana, X. D., & Moreno-Amich, R. (2004). Succession of the animal community in a Mediterranean temporary pond. *Journal of the North American Benthological Society*, *23*(1), 29–49. https://doi.org/10.1899/0887-3593(2004)023<0029:SOTACI>2.0.CO;2

Boudreau, P. R., & Dickie, L. M. (1992). Biomass spectra of aquatic ecosystems in relation to fisheries yield. *Canadian Journal of Fisheries and Aquatic Sciences*, *49*(8), 1528–1538. https://doi.org/10.1139/f92-169

Bozdogan, H. (1987). Model selection and Akaike's Information Criterion (AIC): The general theory and its analytical extensions. *Psychometrika*, *52*(3), 345–370. https://doi.org/10.1007/BF02294361

Braun, L. M., Brucet, S., & Mehner, T. (2021). Top-down and bottom-up effects on zooplankton size distribution in a deep stratified lake. *Aquatic Ecology*, *55*(2), 527–543. https://doi.org/10.1007/s10452-021-09843-8

Brendonck & Williams. (2000). Biodiversity in wetlands of dry regions (drylands). *Biodiversity in Wetlands: Assessment, Function and Conservation*, *1*, 181–194.

Brett, J. R., & Groves, T. D. D. (1979). Physiological energetics. In *Fish Physiology*, 8(6), 280-352.

https://doi.org/10.1016/S1546-5098(08)60029-1

Britton, R. H., & Crivelli, A. J. (1993). Wetlands of southern Europe and North Africa: Mediterranean wetlands. *Wetlands of the World: Inventory, Ecology and Management. Vol. I*, 129–194. https://doi.org/10.1007/978-94-015-8212-4_6

Brönmark, C. (1985). Freshwater snail diversity: effects of pond area, habitat heterogeneity and isolation. *Oecologia*, 67(1), 127-131. https://doi.org/10.1007/BF00378463

Brönmark, C., Herrmann, J., Malmqvist, B., Otto, C., & Sjöström, P. (1984). Animal community structure as a function of stream size. *Hydrobiologia*, 112(1), 73-79. https://doi.org/10.1007/BF00007669

Brown, J. H., Gillooly, J. F., Allen, A. P., Savage, V. M., & West, G. B. (2004). Toward a metabolic theory of ecology. *Ecology*, *85*(7), 1771–1789. https://doi.org/10.1890/03-9000

Brucet, S., Arranz, I., Mehner, T., Argillier, C., Beklioğlu, M., Benejam, L., Boll, T., Holmgren, K., Lauridsen, T. L., Svenning, J. C., Winfield, I. J., & Jeppesen, E. (2018). Size diversity and species diversity relationships in fish assemblages of Western Palearctic lakes. *Ecography*, 41(7), 1064-1076. https://doi.org/10.1111/ecog.03255

Brucet, S., Boix, D., Gascón, S., Sala, J., Quintana, X. D., Badosa, A., Søndergaard, M., Lauridsen, T. L., & Jeppesen, E. (2009). Species richness of crustacean zooplankton and trophic structure of brackish lagoons in contrasting climate zones: North temperate Denmark and Mediterranean Catalonia (Spain). *Ecography*, *32*(4), 692–702. https://doi.org/10.1111/j.1600-0587.2009.05823.x

Brucet, S., Boix, D., López-Flores, R., Badosa, A., Moreno-Amich, R., & Quintana, X. D. (2005). Zooplankton structure and dynamics in permanent and temporary Mediterranean salt marshes: Taxon-based and size-based approaches. *Archiv Fur Hydrobiologie*, *162*(4), 535–555. https://doi.org/10.1127/0003-9136/2005/0162-0535

Brucet, S., Boix, D., López-Flores, R., Badosa, A., & Quintana, X. D. (2006). Size and species diversity of zooplankton communities in fluctuating Mediterranean salt marshes. *Estuarine, Coastal and Shelf Science*, *67*(3), 424–432. https://doi.org/10.1016/j.ecss.2005.11.016

Brucet, S., Boix, D., Nathansen, L. W., Quintana, X. D., Jensen, E., Balayla, D., Meerhoff, M., & Jeppesen, E. (2012). Effects of temperature, salinity and fish in structuring the macroinvertebrate community in shallow lakes: Implications for effects of climate change. *PLoS ONE*, *7*(2) e30877. https://doi.org/10.1371/journal.pone.0030877

Brucet, S., Boix, D., Quintana, X. D., Jensen, E., Nathansen, L. W., Trochine, C., Meerhoff, M., Gascó, S., & Jeppesen, E. (2010). Factors influencing zooplankton size structure at contrasting temperatures in coastal shallow lakes: Implications for effects of climate change. *Limnology and Oceanography*, *55*(4), 1697–1711. https://doi.org/10.4319/lo.2010.55.4.1697

Brucet, S., Compte, J., Boix, D., López-Flores, R., & Quintana, X. D. (2008). Feeding of nauplii, copepodites and adults of Calanipeda aquaedulcis (Calanoida) in Mediterranean salt marshes. *Marine Ecology Progress Series*, *355*, 183–191. https://doi.org/10.3354/meps07225

Brucet, S., Pédron, S., Mehner, T., Lauridsen, T. L., Argillier, C., Winfield, I. J., Volta, P., Emmrich, M., Hesthagen, T., Holmgren, K., Benejam, L., Kelly, F., Krause, T., Palm, A., Rask, M., & Jeppesen, E. (2013). Fish diversity in European lakes: Geographical factors dominate over anthropogenic pressures. *Freshwater Biology*, 58(9), 1779-1793. https://doi.org/10.1111/fwb.12167

Brucet, S., Quintana, X. D., Moreno-Amich, R., & Boix, D. (2005). Changes in the shape of zooplankton biomass-size spectra at ecological scaling in a fluctuating ecosystem (Empordà Wetlands, NE Spain). *Vie et Milieu*, *55*(1), 31–40.

Brucet, S., Tavşanoğlu, Ü. N., Özen, A., Levi, E. E., Bezirci, G., Çakıroğlu, A. İ., Jeppesen, E., Svenning, J. C., Ersoy, Z., & Beklioğlu, M. (2017). Size-based interactions across trophic levels in food webs of shallow Mediterranean lakes. *Freshwater Biology*, 62(11), 1819-1830. https://doi.org/10.1111/fwb.12997

Burghelea, C. I., Zaharescu, D. G., & Palanca-Soler, A. (2010). Dietary overview of Pelophylax perezi from monegros rice fields (northeast Spain). *Herpetological Journal*, *20*(4), 219–224.

Busnardo, M., Gesberg, R. M., Langis, R., Zedles, J., & Sinicrope, T. (1992). Nitrogen and phosphorous removal by wetland mesocosm subjected to different hydroperiods. *Ecological Engineering*, *1*, 287–307. https://doi.org/10.1016/0925-8574(92)90013-R

Caiola, N., & De Sostoa, A. (2005). Possible reasons for the decline of two native toothcarps in the Iberian Peninsula: Evidence of competition with the introduced Eastern mosquitofish. *Journal of Applied Ichthyology*, 21(4), 358-363. https://doi.org/10.1111/j.1439-0426.2005.00684.x

Carmona-Catot, G., Magellan, K., & García-Berthou, E. (2013). Temperature-Specific Competition between Invasive Mosquitofish and an Endangered Cyprinodontid Fish. *PLoS ONE*, *8*(1). https://doi.org/10.1371/journal.pone.0054734

Casas, J. J., Sánchez-Oliver, J. S., Sanz, A., Furné, M., Trenzado, C., Juan, M., Paracuellos, M., Suárez, M. D., Fuentes, F., Gallego, I., Gil, C., & Ramos-Miras, J. J. (2011). The paradox of the conservation of an endangered fish species in a Mediterranean region under agricultural intensification. *Biological Conservation*, 144(1), 253-262. https://doi.org/10.1016/j.biocon.2010.08.023

CEN. (2005). EVS-EN 14757:2005. Water quality - Sampling of fish with multi-mesh gillnets. *En 14757:2005*.

Céréghino, R., Biggs, J., Oertli, B., & Declerck, S. (2008). The ecology of European ponds: Defining the characteristics of a neglected freshwater habitat. *Hydrobiologia*, *597*(1), 1–6. https://doi.org/10.1007/s10750-007-9225-8

Céréghino, R., Boix, D., Cauchie, H. M., Martens, K., & Oertli, B. (2014). The ecological role of ponds in a changing world. *Hydrobiologia, 723*(1), 1–6. https://doi.org/10.1007/s10750-013-1719-y

Chaichana, R., Leah, R., & Moss, B. (2011). Conservation of pond systems: A case study of intractability, Brown Moss, UK. *Hydrobiologia*, *664*(1), 17–33. https://doi.org/10.1007/s10750-010-0579-y

Chang, C. W., Miki, T., Shiah, F. K., Kao, S. J., Wu, J. T., Sastri, A. R., & Hsieh, C. H. (2014). Linking secondary structure of individual size distribution with nonlinear size-trophic level relationship in food webs. *Ecology*, 95(4), 897-909. https://doi.org/10.1890/13-0742.1

Clavero, M., Blanco-Garrido, F., & Prenda, J. (2006). Monitoring small fish populations in streams: A comparison of four passive methods. *Fisheries Research*, *78*(2–3), 243–251. https://doi.org/10.1016/j.fishres.2005.11.016

Cognetti, G., & Maltagliati, F. (2000). Biodiversity and adaptive mechanisms in brackish water fauna. *Marine Pollution Bulletin*, *40*(1), 7–14. https://doi.org/10.1016/S0025-326X(99)00173-3

Cohen, J. E., Pimm, S. L., Yodzis, P., & Saldana, J. (1993). Body Sizes of Animal Predators and Animal Prey in Food Webs. *The Journal of Animal Ecology*, *62*(1), 67. https://doi.org/10.2307/5483

Collos, Y., Husseini-Ratrema, J., Bec, B., Vaquer, A., Thong, L. H., Rougier, C., Pons, V., & Souchu, P. (2005). Pheopigment dynamics, zooplankton grazing rates and the autumnal ammonium peak in a Mediterranean lagoon. *Hydrobiologia*, 550(1), 83-93. https://doi.org/10.1007/s10750-005-4365-1

Comin, F. A., & Valiela, I. (1993). On the Controls of Phytoplankton Abundance and Production in Coastal Lagoons, 895-906. *Journal of Coastal Research*. https://doi.org/10.2307/4298149

Compte, J., Gascón, S., Quintana, X. D., & Boix, D. (2010). Top-predator effects of jellyfish Odessia maeotica in Mediterranean salt marshes. *Marine Ecology Progress Series*, *402*(1992), 147–159. https://doi.org/10.3354/meps08453

Compte, J., Gascón, S., Quintana, X. D., & Boix, D. (2011). Fish effects on benthos and plankton in a Mediterranean salt marsh. *Journal of Experimental Marine Biology and Ecology*, *409*(1–2), 259–266. https://doi.org/10.1016/j.jembe.2011.09.005

Compte, J., Gascón, S., Quintana, X. D., & Boix, D. (2012). The effects of small fish presence on a species-poor community dominated by omnivores: Example of a size-based trophic cascade. *Journal of Experimental Marine Biology and Ecology*, *418–419*, 1–11. https://doi.org/10.1016/j.jembe.2012.03.004

Cooper, S. D. (1983). Selective predation of cladocerans by common pond insects. *Canadian Journal of Zoology*, *61*(4), 879–886. https://doi.org/10.1139/z83-115

Cousins, S. H. (1980). A trophic continuum derived from plant structure, animal size and a detritus cascade. *Journal of Theoretical Biology*, *82*(4), 607–618. https://doi.org/10.1016/0022-5193(80)90181-2

Cramer, W., Guiot, J., Fader, M., Garrabou, J., Gattuso, J. P., Iglesias, A., Lange, M. A., Lionello, P., Llasat, M. C., Paz, S., Peñuelas, J., Snoussi, M., Toreti, A., Tsimplis, M. N., & Xoplaki, E. (2018). Climate change and interconnected risks to sustainable development in the Mediterranean. *Nature Climate Change*, *8*(11), 972–980. https://doi.org/10.1038/s41558-018-0299-2

Crivelli, A. (2006). *Aphanius iberus. The IUCN Red List of Threatened Species 2006: e.T1846A8299534*. The IUCN Red List of Threatened Species 2006.

Crivelli, A., & Boy, V. (1987). The diet of the mosquitofish Gambusia affinis (Baird & Girard) (Poecilliidae) in Mediterranean France. *Revue d'écologie*, *42(4)*, 421–435.

Daft Logic. (2016). *Google Maps Area Calculator Tool*. Www.Daftlogic.Com.

Davies, B. R., Biggs, J., Williams, P. J., Lee, J. T., & Thompson, S. (2008). A comparison of the catchment sizes of rivers, streams, ponds, ditches and lakes: Implications for protecting aquatic biodiversity in an agricultural landscape. *Hydrobiologia*, *597*(1), 7–17. https://doi.org/10.1007/s10750-007-9227-6

De Meester, L., Declerck, S., Stoks, R., Louette, G., Van De Meutter, F., De Bie, T., Michels, E., & Brendonck, L. (2005). Ponds and pools as model systems in conservation biology, ecology and evolutionary biology. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *15*(6), 715–725. https://doi.org/10.1002/aqc.748

De Sostoa, A. (1983). *Las comunidades de peces del delta del Ebro. PhD Thesis, University of Barcelona: 622 pp.*

Declerck, S., De Meester, L., Podoor, N., & Conde-Porcuna, J. (1997). The relevance of size efficiency to biomanipulation theory: a field test under hypertrophic conditions. *Hydrobiologia*, 360: 265–275.

Declerck, S., De Bie, T., Ercken, D., Hampel, H., Schrijvers, S., Van Wichelen, J., Gillard, V., Mandiki, R., Losson, B., Bauwens, D., Keijers, S., Vyverman, W., Goddeeris, B., De meester, L., Brendonck, L., & Martens, K. (2006). Ecological characteristics of small farmland ponds: Associations with land use practices at multiple spatial scales. *Biological Conservation*, *131*(4), 523–532. https://doi.org/10.1016/j.biocon.2006.02.024

Della Bella, V., Bazzanti, M., & Chiarotti, F. (2005). Macroinvertebrate diversity and conservation status of Mediterranean ponds in Italy: Water permanence and mesohabitat influence. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *15*(6), 583–600. https://doi.org/10.1002/aqc.743

Demestre, M., Roig, A., De Sostoa, A., & De Sostoa, F. (1977). Contribució a l'estudi de la ictiofauna continental del delta de I'Ebre. *Treballs de l'Institut Catalá d'Historia Natural*, *8*, 145–226.

DeMott, W. R., Gulati, R. D., & Van Donk, E. (2001). Daphnia food limitation in three hypereutrophic Dutch lakes: Evidence for exclusion of large-bodied species by interfering filaments of cyanobacteria. *Limnology and Oceanography*, *46*(8), 2054–2060. https://doi.org/10.4319/lo.2001.46.8.2054

Dickie, L.M., Kerr, S.R., Bodreau, P.R. (1987). Size-Dependent Processes Underlying Regularities in Ecosystem Structure. *Ecological Monographs, 57*(3), 233–250.

Dimitriou, E., Karaouzas, I., Skoulikidis, N., & Zacharias, I. (2006). Assessing the environmental status of Mediterranean temporary ponds in Greece. *Annales de Limnologie*, *42*(1), 33–41. https://doi.org/10.1051/limn/2006004

Doadrio, I. (2001). *Atlas y libro rojo de los peces continentals de España*. 364 p. *Madrid: MNCN-CSIC.*

Doadrio, I., Carmona, J. A., & Fernández-Delgado, C. (2002). Morphometric study of the Iberian Aphanius (Actinopterygii, Cyprinodontiformes), with description of a new species. *Folia Zoologica*, 51(1), 67-80.

Dodson, S., & Brooks, J. L. (1965). Predation , Body Size , and Composition of Plankton The effect of a marine planktivore on lake plankton. *Science*, *150*(3692), 28–35. https://doi.org/10.1126/science.150.3692.28

Dodson, S. (1991). Species richness of crustacean zooplankton in European lakes of different sizes. *Verhandlungen Der Internationalen Vereinigung Für Theoretische Und Angewandte Limnologie*, 24(2),1223-1229. https://doi.org/10.1096/fj.03-1291fje

Dodson, S. (1992). Predicting crustacean zooplankton species richness. *Limnology and Oceanography*, 37(4), 848-856. https://doi.org/10.4319/lo.1992.37.4.0848

Dossena, M., Yvon-Durocher, G., Grey, J., Montoya, J. M., Perkins, D. M., Trimmer, M., & Woodward, G. (2012). Warming alters community size structure and ecosystem functioning. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1740), 3011–3019. https://doi.org/10.1098/rspb.2012.0394

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A. H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. In *Biological Reviews of the Cambridge Philosophical Society*, 81(2), 163-182. https://doi.org/10.1017/S1464793105006950

Dumont, H. J., Van de Velde, I., & Dumont, S. (1975). The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. *Oecologia*, *19*(1), 75–97. https://doi.org/10.1007/BF00377592

Eadie, J. M. A., Hurly, T. A., Montgomerie, R. D., & Teather, K. L. (1986). Lakes and rivers as islands: species-area relationships in the fish faunas of Ontario. *Environmental Biology of Fishes*, 15(2), 81-89. https://doi.org/10.1007/BF00005423

Emmerson, M. C., & Raffaelli, D. (2004). Predator-prey body size, interaction strength and the stability of a real food web. *Journal of Animal Ecology*, *73*(3), 399–409. https://doi.org/10.1111/j.0021-8790.2004.00818.x

Emmrich, M., Brucet, S., Ritterbusch, D., & Mehner, T. (2011). Size spectra of lake fish assemblages: Responses along gradients of general environmental factors and intensity of lake-use. *Freshwater Biology*, 56(11), 2316-2333. https://doi.org/10.1111/j.1365-2427.2011.02658.x

Emmrich, M., Pédron, S., Brucet, S., Winfield, I. J., Jeppesen, E., Volta, P., Argillier, C., Lauridsen, T. L., Holmgren, K., Hesthagen, T., & Mehner, T. (2014). Geographical patterns in the body-size structure of European lake fish assemblages along abiotic and biotic gradients. *Journal of Biogeography*, *41*(12), 2221–2233. https://doi.org/10.1111/jbi.12366

Erdfelder, E., Faul, F., Buchner, A., & Lang, A. G. (2009). Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods*, *41*(4), 1149–1160. https://doi.org/10.3758/BRM.41.4.1149

Ersoy, Z., Jeppesen, E., Sgarzi, S., Arranz, I., Cañedo-Argüelles, M., Quintana, X. D., Landkildehus, F., Lauridsen, T. L., Bartrons, M., & Brucet, S. (2017). Size-based interactions and trophic transfer efficiency are modified by fish predation and cyanobacteria blooms in Lake Mývatn, Iceland. *Freshwater Biology*, *62*(11), 1942–1952. https://doi.org/10.1111/fwb.13039

Fernández-Aláez, M., & Fernández-Aláez, C. (2010). Effects of the intense summer desiccation and the autumn filling on the water chemistry in some Mediterranean ponds. *Limnetica*, *29*(1), 59–74.

https://doi.org/10.23818/limn.29.05

Finlay, K., Beisner, B. E., Patoine, A., & Pinel-Alloul, B. (2007). Regional ecosystem variability drives the relative importance of bottom-up and top-down factors for zooplankton size spectra. *Canadian Journal of Fisheries and Aquatic Sciences*, *64*(3), 516–529. https://doi.org/10.1139/F07-028

Fischlin, A., Midgley, G. F., Price, J. T., Leemans, R., Gopal, B., Turley, C., Velichko, A. A. (2007). Ecosystems, their properties, goods and services.

Fletcher, T. D. (2012). QuantPsyc: quantitative psychology tools. *R Package Version*.

Florencio, M., Díaz-Paniagua, C., Gómez-Rodríguez, C., & Serrano, L. (2014). Biodiversity patterns in a macroinvertebrate community of a temporary pond network. *Insect Conservation and Diversity*, *7*(1), 4–21. https://doi.org/10.1111/icad.12029

Fois, M., Cuena-Lombraña, A., & Bacchetta, G. (2021). Knowledge gaps and challenges for conservation of Mediterranean wetlands: Evidence from a comprehensive inventory and literature analysis for Sardinia. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *31*(9), 2621–2631. https://doi.org/10.1002/aqc.3659

Fouilland, E. (1996). *for population assessment of river Macrobrachium (Crustacea : Decapoda)*. *28*(96), 343–351.

Fox, J. W. (2004). Modelling the joint effects of predator and prey diversity on total prey biomass. *Journal of Animal Ecology*, *73*(1), 88–96. https://doi.org/10.1111/j.1365-2656.2004.00784.x

Franco, A., Pérez-Ruzafa, A., Drouineau, H., Franzoi, P., Koutrakis, E. T., Lepage, M., Verdiell-Cubedo, D., Bouchoucha, M., López-Capel, A., Riccato, F., Sapounidis, A., Marcos, C., Oliva-Paterna, F. J., Torralva-Forero, M., & Torricelli, P. (2012). Assessment of fish assemblages in coastal lagoon habitats: Effect of sampling method. *Estuarine, Coastal and Shelf Science*, 112, 115-125. https://doi.org/10.1016/j.ecss.2011.08.015

Franco, A., Torricelli, P., & Franzoi, P. (2009). A habitat-specific fish-based approach to assess the ecological status of Mediterranean coastal lagoons. *Marine Pollution Bulletin*, 58(11), 1704-1717. https://doi.org/10.1016/j.marpolbul.2009.06.016

Frascari, F., Matteucci, G., & Giordano, P. (2002). Evaluation of a eutrophic coastal lagoon ecosystem from the study of bottom sediments, in Nutrients and Eutrophication in Estuaries and coastal waters. *Hydrobiologia*, 387-401. https://doi.org/10.1023/A:1020399627807

Fryer, G. (1985). Crustacean diversity in relation to the size of water bodies: some facts and problems. *Freshwater Biology*, 15(3), 347-361. https://doi.org/10.1111/j.1365-2427.1985.tb00206.x

Gaedke, U. (1992). The size distribution of plankton biomass in a large lake and its seasonal variability. *Limnology and Oceanography*, *37*(6), 1202–1220. https://doi.org/10.4319/lo.1992.37.6.1202

Gaedke, U. (1995). A comparison of whole-community and ecosystem approaches (biomass size distributions, food web analysis, network analysis, simulation models) to study the structure, function and regulation of pelagic food webs. *Journal of Plankton Research*, *17*(6), 1273–1305. https://doi.org/10.1093/plankt/17.6.1273

Gaedke, U., Seifried, A., & Adrian, R. (2004). Biomass Size Spectra and Plankton Diversity in a Shallow Eutrophic Lake. *International Review of Hydrobiology*, *89*(1), 1–20. https://doi.org/10.1002/iroh.200310661

Garcia-Berthou, E. (1999). Food of introduced mosquitofish: ontogenetic diet shift and prey selection. *Journal of Fish Biology*, *55(1)*, 135–147. https://doi.org/10.1111/j.1095-8649.1999.tb00663.x

García-Berthou, E., & Moreno-Amich, R. (1999). Ecología y conservación del fartet (*Lebias ibera*) en las marismas del Ampurdán (Cataluña). In *Peces ciprinodóntidos ibéricos. Fartet y Samaruc*, 50(7), 1394-1399.

García-Berthou, E., & Moreno-Amich, R. (1991). New records of Aphanius iberus (Pisces: Cyprinodontidae) and review of the geographical distribution of Cyprinodontiform fishes in Catalonia (NE-Spain). *Scientia Gerundensis*, *76*, 69–76. http://invasiber.org/EGarcia/papers/sg91.pdf

García-Berthou, E., & Moreno-Amich, R. (1993). Multivariate analysis of covariance in morphometric studies of the reproductive cycle. *Canadian Journal of Fisheries and Aquatic Sciences*, 50(7), 1394-1399. https://doi.org/10.1139/f93-159.

García-Berthou, E., & Moreno-Amich, R. (1992). Age and growth of an Iberian cyprinodont, Aphanius iberus (Cuv. & Val.), in its most northerly population. *Journal of Fish Biology*, 40(6), 929-937. https://doi.org/10.1111/j.1095-8649.1992.tb02638.x

García-Comas, C., Sastri, A. R., Ye, L., Chang, C. Y., Lin, F. S., Su, M. S., Gong, G. C., & Hsieh, C. H. (2016). Prey size diversity hinders biomass trophic transfer and predator size diversity promotes it in Planktonic Communities. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1824). https://doi.org/10.1098/rspb.2015.2129

García-Girón, J., Fernández-Aláez, M., & Fernández-Aláez, C. (2019). Redundant or complementary? Evaluation of different metrics as surrogates of macrophyte biodiversity patterns in Mediterranean ponds. *Ecological Indicators*, *101*(January), 614–622. https://doi.org/10.1016/j.ecolind.2019.01.062

Gardner, J. L., Amano, T., Backwell, P. R. Y., Ikin, K., Sutherland, W. J., & Peters, A. (2014). Temporal patterns of avian body size reflect linear size responses to broadscale environmental change over the last 50 years.

Journal of Avian Biology, *45*(6), 529–535. https://doi.org/10.1111/jav.00431

Gardner, J. L., Peters, A., Kearney, M. R., Joseph, L., & Heinsohn, R. (2011). Declining body size: A third universal response to warming? *Trends in Ecology and Evolution*, *26*(6), 285–291. https://doi.org/10.1016/j.tree.2011.03.005

Garzke, J., Ismar, S. M. H., & Sommer, U. (2015). Climate change affects low trophic level marine consumers: warming decreases copepod size and abundance. *Oecologia*, *177*(3), 849–860. https://doi.org/10.1007/s00442-014-3130-4

Gascón, Boix, D., Sala, J., & Quintana, X. D. (2009). Patterns in size and species diversity of benthic macroinvertebrates in mediterranean salt marshes. *Marine Ecology Progress Series*, *391*(2008), 21–32. https://doi.org/10.3354/meps08151

Gascón, S., MacHado, M., Sala, J., Cancela Da Fonseca, L., Cristo, M., & Boix, D. (2012). Spatial characteristics and species niche attributes modulate the response by aquatic passive dispersers to habitat degradation. *Marine and Freshwater Research*, *63*(3), 232–245. https://doi.org/10.1071/MF11160

Gascón, S., Boix, D., & Sala, J. (2009). Are different biodiversity metrics related to the same factors? A case study from Mediterranean wetlands. *Biological Conservation*, *142*(11), 2602–2612. https://doi.org/10.1016/j.biocon.2009.06.008

Gascón, S., Boix, D., Sala, J., & Quintana, X. D. (2005). Variability of benthic assemblages in relation to the hydrological pattern in Mediterranean salt marshes (Empordà wetlands, NE Iberian Peninsula). *Archiv Für Hydrobiologie*, *163*(2), 163–181. https://doi.org/10.1127/0003-9136/2005/0163-0163

Geijzendorffer, I., Chazee, L., Gaget, E., Galewski, T., Guelmami, A., & Perennou, C. (2018). Mediterranean wetland outlook 2: solutions for sustainable Mediterranean wetlands. *Secretariat of the Ramsar Convention*.

Gelós, M., Teixeira-de Mello, F., Goyenola, G., Iglesias, C., Fosalba, C., García-Rodríguez, F., Pacheco, J. P., García, S., & Meerhoff, M. (2010). Seasonal and diel changes in fish activity and potential cascading effects in subtropical shallow lakes with different water transparency. *Hydrobiologia*, 646(1), 173-185. https://doi.org/10.1007/s10750-010-0170-6

Gelwick, F. P., Akin, S., Arrington, D. A., & Winemiller, K. O. (2001). Fish assemblage structure in relation to environmental variation in a Texas Gulf coastal wetland. *Estuaries*, *24*(2), 285–296. https://doi.org/10.2307/1352952

Ghosn, D., Vogiatzakis, I. N., Kazakis, G., Dimitriou, E., Moussoulis, E., Maliaka, V., & Zacharias, I. (2010). Ecological changes in the highest temporary pond of western Crete (Greece): Past, present and future. *Hydrobiologia*, *648*(1), 3–18. https://doi.org/10.1007/s10750-010-0143-9

Green, A. J., Fuentes, C., Moreno-Ostos, E., & Rodrigues Da Silva, S. L. (2005). Factors influencing cladoceran abundance and species richness in brackish lakes in Eastern Spain. *Annales de Limnologie*, *41*(2), 73–81. https://doi.org/10.1051/limn/2005010

Guidi, L., Stemmann, L., Jackson, G. A., Ibanez, F., Claustre, H., Legendre, L., Picheral, M., & Gorsky, G. (2009). Effects of phytoplankton community on production, size and export of large aggregates: A world-ocean analysis. *Limnology and Oceanography*, *54*(6), 1951–1963. https://doi.org/10.4319/lo.2009.54.6.1951

Haiahem, D., Touati, L., Baaziz, N., Samraoui, F., Alfarhan, A. H., & Samraoui, B. (2017). Impact of eastern mosquitofish, Gambusia holbrooki, on temporary ponds: insights on how predation may structure zooplankton communities. *Zoology and Ecology*, 27(2), 124-132. https://doi.org/10.1080/21658005.2017.1337372

Hall, D. J., Threlkeld, S. T., Burns, C. W., & Crowley, P. H. (1976). The Size-Efficiency Hypothesis and the Size Structure of Zooplankton Communities. *Annual Review of Ecology and Systematics*, *7*(1), 177–208. https://doi.org/10.1146/annurev.es.07.110176.001141

Halpern, B. S., Walbridge, S., Selkoe, K. A., Kappel, C. V., Micheli, F., D'Agrosa, C., Bruno, J. F., Casey, K. S., Ebert, C., Fox, H. E., Fujita, R., Heinemann, D., Lenihan, H. S., Madin, E. M. P., Perry, M. T., Selig, E. R., Spalding, M., Steneck, R., & Watson, R. (2008). A global map of human impact on marine ecosystems. *Science*, *319*(5865), 948–952. https://doi.org/10.1126/science.1149345

Hampton, S. E., & Gilbert, J. J. (2001). Observations of insect predation on rotifers. *Hydrobiologia*, *446–447*, 115–121. https://doi.org/10.1023/A:1017543121353

Hampton, S. E., Gilbert, J. J., & Burns, C. W. (2000). Direct and indirect effects of juvenile Buenoa macrotibialis (Hemiptera: Notonectidae) on the zooplankton of a shallow pond. *Limnology and Oceanography*, *45*(4), 1006–1012. https://doi.org/10.4319/lo.2000.45.4.1006

Hart, B. T., Bailey, P., Edwards, R., Hortle, K., James, K., McMahon, A., Meredith, C., & Swadling, K. (1990). Effects of salinity on river, stream and wetland ecosystems in Victoria, Australia. *Water Research*, *24*(9), 1103–1117. https://doi.org/10.1016/0043-1354(90)90173-4

Havens, K. E., Pinto-Coelho, R. M., Beklioğlu, M., Christoffersen, K. S., Jeppesen, E., Lauridsen, T. L., Mazumder, A., Méthot, G., Alloul, B. P., Tavşanoğlu, U. N., Erdoğan, Ş., & Vijverberg, J. (2015). Temperature effects on body size of freshwater crustacean zooplankton from Greenland to the tropics. *Hydrobiologia*, *743*(1), 27–35. https://doi.org/10.1007/s10750-014-2000-8

Holmgren, K., & Appelberg, M. (2000). Size structure of benthic freshwater fish communities in relation to environmental gradients. *Journal of Fish Biology*, 57(5), 1312-1330. https://doi.org/10.1006/jfbi.2000.1395

Jennings, S., & Blanchard, J. L. (2004). Fish abundance with no fishing: Predictions based on macroecological theory. *Journal of Animal Ecology*, *73*(4), 632–642. https://doi.org/10.1111/j.0021-8790.2004.00839.x

Jensen, E., Brucet, S., Meerhoff, M., Nathansen, L., & Jeppesen, E. (2010). Community structure and diel migration of zooplankton in shallow brackish lakes: Role of salinity and predators. *Hydrobiologia*, *646*(1), 215–229. https://doi.org/10.1007/s10750-010-0172-4

Jeppesen, E., Jensen, J. P., Lauridsen, T. L., Amsinck, S. L., Christoffersen, K., Søndergaard, M., & Mitchell, S. F. (2003). Sub-fossils of cladocerans in the surface sediment of 135 lakes as proxies for community structure of zooplankton, fish abundance and lake temperature. *Hydrobiologia*, 491(1), 321–330. https://doi.org/10.1023/A:1024488525225

Jeppesen, E., Jensen, J. P., Sondergaard, M., Lauridsen, T., & Landkildehus, F. (2000). Trophic structure, species richness and biodiversity in Danish lakes: Changes along a phosphorus gradient. *Freshwater Biology*, *45*(2), 201–218. https://doi.org/10.1046/j.1365-2427.2000.00675.x

Jeppesen, E., Meerhoff, M., Jacobsen, B. A., Hansen, R. S., Søndergaard, M., Jensen, J. P., Lauridsen, T. L., Mazzeo, N., & Branco, C. W. C. (2007). Restoration of shallow lakes by nutrient control and biomanipulation - The successful strategy varies with lake size and climate. *Hydrobiologia*, *581*(1), 269–285. https://doi.org/10.1007/s10750-006-0507-3

Jeppesen, E., Peder Jensen, J., Søndergaard, M., Lauridsen, T., Junge Pedersen, L., & Jensen, L. (1997). Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. *Shallow Lakes '95*, 151–164. https://doi.org/10.1007/978-94-011-5648-6_17

Jeppesen, E., Sondergaard, M., Kanstrup, E., Petersen, B., Eriksen, R. B., Hammershoj, M., Mortensen, E., Jensen, J. P., & Have, A. (1994). Does the impact of nutrients on the biological structure and function of brakish and freshwater lakes differ? *Hydrobiologia*, *275/276*, 15–30.

Jinks, K. I., Brown, C. J., Rasheed, M. A., Scott, A. L., Sheaves, M., York, P. H., & Connolly, R. M. (2019). Habitat complexity influences the structure of food webs in Great Barrier Reef seagrass meadows. *Ecosphere*, *10*(11). https://doi.org/10.1002/ecs2.2928

Kenk, R. (1949). The animal life of temporary and permanent ponds in southern Michigan. *Miscellaneous Publications of Museum of Zoology, University of Michigan*, *71*(71), 1–73.

Kerr, S. ., Dickie, L. . (2001). The biomass spectrum. In *Columbia University Press, New York, New York, USA*.

Kreutzer, C., & Lampert, W. (1999). Exploitative competition in differently sized Daphnia species: A mechanistic explanation. *Ecology*, *80*(7), 2348–2357. https://doi.org/10.1890/0012-9658(1999)080[2348:ecidsd]2.0.co;2

Kristensen, P., & Globevnik, L. (2014). European small water bodies. *Biology and Environment*, *114B*(3), 281–287. https://doi.org/10.3318/BIOE.2014.13

Lahr, J., Diallo, A. O., Ndour, K. B., Badji, A., & Diouf, P. S. (1999). *Phenology of invertebrates living in a sahelian temporary pond*. 189–205.

Lake, P. S., Bayly, I. A. E., & Morton, D. W. (1989). The phenology of a temporary pond in western Victoria, Australia, with special reference to invertebrate succession. *Archiv Für Hydrobiologie*, 115 (2), 171–202.

Leonardos, I., & Sinis, A. (1998). Reproductive strategy of Aphanius fasciatus Nardo, 1827 (Pisces: Cyprinodontidae) in the Mesolongi and Etolikon lagoons (W. Greece). *Fisheries Research*, 35(3), 171-181. https://doi.org/10.1016/S0165-7836(98)00082-4

Litchman, E., & Klausmeier, C. A. (2008). Trait-Based Community Ecology of Phytoplankton. *Annual Review of Ecology, Evolution, and Systematics*, *39*(1), 615–639. https://doi.org/10.1146/annurev.ecolsys.39.110707.173549

López-Flores, R., Boix, D., Badosa, A., Brucet, S., & Quintana, X. D. (2006). Pigment composition and size distribution of phytoplankton in a confined Mediterranean salt marsh ecosystem. *Marine Biology*, *149*(6), 1313–1324. https://doi.org/10.1007/s00227-006-0273-9

López-Flores, R., Boix, D., Badosa, A., Brucet, S., & Quintana, X. D. (2009). Environmental factors affecting bacterioplankton and phytoplankton dynamics in confined Mediterranean salt marshes (NE Spain). *Journal of Experimental Marine Biology and Ecology*, *369*(2), 118–126. https://doi.org/10.1016/j.jembe.2008.11.003

López-Flores, R., Garcés, E., Boix, D., Badosa, A., Brucet, S., Masó, M., & Quintana, X. D. (2006). Comparative composition and dynamics of harmful dinoflagellates in Mediterranean salt marshes and nearby external marine waters. *Harmful Algae*, *5*(6), 637–648. https://doi.org/10.1016/j.hal.2006.01.001

López-Flores, R., Quintana, X. D., Romaní, A. M., Bañeras, L., Ruiz-Rueda, O., Compte, J., Green, A. J., & Egozcue, J. J. (2014). A compositional analysis approach to phytoplankton composition in coastal

Mediterranean wetlands: Influence of salinity and nutrient availability. *Estuarine, Coastal and Shelf Science*, *136*, 72–81. https://doi.org/10.1016/j.ecss.2013.11.015

López-Flores, R., Quintana, X. D., Salvadó, V., Hidalgo, M., Sala, L., & Moreno-Amich, R. (2003). Comparison of nutrient and contaminant fluxes in two areas with different hydrological regimes (Empordà Wetlands, NE Spain). *Water Research*, *37*(12), 3034–3046. https://doi.org/10.1016/S0043-1354(03)00109-X

Lorenz, J. J., & Serafy, J. E. (2006). Subtroprical wetland fish assemblages and changing salinity regimes: Implications for everglades restoration. *Hydrobiologia*, *569*(1), 401–422. https://doi.org/10.1007/s10750-006-0145-9

Lowe, S., Browne, M., Boudjelas, S., & De Poorter, M. (2000). 100 of The World Worst Invasive Alien Species. A Selection from The Global Invasive Species Database. The Invasive Species Specialist Group, SSC, IUCN,. *Hollands Printing Ltd, New Zealand*.

Lumbreras, A., Marques, J. T., Belo, A. F., Cristo, M., Fernandes, M., Galioto, D., Machado, M., Mira, A., Sá-Sousa, P., Silva, R., Sousa, L. G., & Pinto-Cruz, C. (2016). Assessing the conservation status of Mediterranean temporary ponds using biodiversity: a new tool for practitioners. *Hydrobiologia*, *782*(1), 187–199. https://doi.org/10.1007/s10750-016-2697-7

MacArthur, R. ., & Wilson, E. . (1967). The theory of island biogeography. *Princeton University Press,*. https://doi.org/10.1086/282697

Mack, R. N., Simberloff, D., Lonsdale, W. M., Evans, H., Clout, M., & Bazzaz, F. A. (2000). Biotic invasions: Causes, epidemiology, global consequences, and control. *Ecological Applications*, 10(3), 689-710. https://doi.org/10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2

Magellan, K., García-Berthou, E. (2016). Experimental evidence for the use of artificial refugia to mitigate the impacts of invasive Gambusia holbrooki on an endangered fish. *Biological Invasions*, 18(3), 873-882. https://doi.org/10.1007/s10530-016-1057-x

Malley, D., Lawrence, S. ., Mativer, M. ., & Findlay, M. W. . (1989). Range of variation in estimates of dry weight for planktonic Crustacea and Rotifera from temperate North American lakes. *Canadian Technical Report of Fisheries Und Aquatic Sciencies*.

Marañón, E., Cermeño, P., Latasa, M., & Tadonléké, R. D. (2012). Temperature, resources, and phytoplankton size structure in the ocean. *Limnology and Oceanography*, *57*(5), 1266–1278. https://doi.org/10.4319/lo.2012.57.5.1266

Marañón, E., Cermeño, P., Latasa, M., & Tadonléké, R. D. (2015). Resource supply alone explains the variability of marine phytoplankton size structure. *Limnology and Oceanography*, *60*(5), 1848–1854. https://doi.org/10.1002/lno.10138

Mariotti, A., Zeng, N., Yoon, J. H., Artale, V., Navarra, A., Alpert, P., & Li, L. Z. X. (2008). Mediterranean water cycle changes: Transition to drier 21st century conditions in observations and CMIP3 simulations. *Environmental Research Letters*, *3*(4). https://doi.org/10.1088/1748-9326/3/4/044001

Martinoy, M., Boix, D., Sala, J., Gascón, S., Gifre, J., Argerich, A., De La Barrera, R., Brucet, S., Badosa, A., López-Flores, R., Méndez, M., Utgé, J. M., & Quintana, X. D. (2006). Crustacean and aquatic insect assemblages in the Mediterranean coastal ecosystems of Empordà wetlands (NE Iberian peninsula). *Limnetica*, *vol. 25, núm. 3, p. 665-682*.

McLusky, D. S., & Elliott, M. (2007). Transitional waters: A new approach, semantics or just muddying the waters? *Estuarine, Coastal and Shelf Science*, *71*(3–4), 359–363. https://doi.org/10.1016/j.ecss.2006.08.025

Mehner, T., Keeling, C., Emmrich, M., Holmgren, K., Argillier, C., Volta, P., Winfield, I. J., & Brucet, S. (2016). Effects of fish predation on density and size spectra of prey fish communities in lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, 73(4), 506-518. https://doi.org/10.1139/cjfas-2015-0034

Mehner, T., Lischke, B., Scharnweber, K., Attermeyer, K., Brothers, S., Gaedke, U., Hilt, S., & Brucet, S. (2018). Empirical correspondence between trophic transfer efficiency in freshwater food webs and the slope of their size spectra. *Ecology*, *99*(6), 1463–1472. https://doi.org/10.1002/ecy.2347

Meyer, E. (1989). The relationship between body length parameters and dry mass in running water invertebrates. *Arch. Hydrobiol.*, 117(2), 191-203. 117:191203.

Mischke, U., Thackeray, S., Dunbar, M., McDonald, C., Carvalho, L., de Hoyos, C., Jarvinen, M., Laplace-Treyture, C., Morabito, G., Skjelbred, B., Lyche Solheim, A., Brierley, B., & Dudley, B. (2012). *WISER deliverable D3.1-4: guidance document on sampling, analysis and counting standards for phytoplankton in lakes*. 1–51. http://nora.nerc.ac.uk/17466/

Møller, T. R., & Rørdam, C. P. (1985). Species numbers of vascular plants in relation to area, isolation and age of ponds in Denmark. *Oikos*, *45*(1), 8–16. https://doi.org/10.2307/3565216

Moreno-Amich, R. (1989). *Els peixos d'aigua dolça i salabrosa. In Els aiguamolls de l'Empord& Aspectes eco16gics, hist6rics i socials del Parc Natural. Caries Vallks, ed. J. Sargatal & J. Felix. Editorial ART-3, Figueres, pp. 241-50.*

Moretti, M., Dias, A. T. C., de Bello, F., Altermatt, F., Chown, S. L., Azcárate, F. M., Bell, J. R., Fournier, B., Hedde, M., Hortal, J., Ibanez, S., Öckinger, E., Sousa, J. P., Ellers, J., & Berg, M. P. (2017). Handbook of protocols for standardized measurement of terrestrial invertebrate functional traits. *Functional Ecology*, *31*(3), 558–567. https://doi.org/10.1111/1365-2435.12776

Moss, B. (1994). Brackish and Fresh-Water Shallow Lakes - Different Systems or Variations on the Same Theme. *Hydrobiologia*, *276*, 1–14. https://doi.org/10.1007/BF00026695

Mouillot, D., Gaillard, S., Aliaume, C., Verlaque, M., Belsher, T., Troussellier, M., & Do Chi, T. (2005). Ability of taxonomic diversity indices to discriminate coastal lagoon environments based on macrophyte communities. *Ecological Indicators*, *5*(1), 1–17. https://doi.org/10.1016/j.ecolind.2004.04.004

Muñoz, A. A., & Ojeda, F. P. (1998). Guild structure of carnivorous intertidal fishes of the Chilean coast: Implications of ontogenetic dietary shifts. *Oecologia*, *114*(4), 563–573. https://doi.org/10.1007/s004420050481

Murry, B. A., & Farrell, J. M. (2014). Resistance of the size structure of the fish community to ecological perturbations in a large river ecosystem. *Freshwater Biology*, *59*(1), 155–167. https://doi.org/10.1111/fwb.12255

Naselli-Flores, L., & Barone, R. (2002). Limnology of a small, temporary water body: the Pond of Santa Rosalia (Sicily, Italy). *SIL Proceedings, 1922-2010*, *28*(4), 1673–1677. https://doi.org/10.1080/03680770.2001.11901906

Oertli, B., Biggs, J., Céréghino, R., Grillas, P., Joly, P., & Lachavanne, J. B. (2005). Conservation and monitoring of pond biodiversity1: Introduction. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *15*(6), 535–540. https://doi.org/10.1002/aqc.752

Oertli, B., Joye, D. A., Castella, E., Juge, R., Cambin, D., & Lachavanne, J. B. (2002). Does size matter? The relationship between pond area and biodiversity. *Biological Conservation*, *104*(1), 59–70. https://doi.org/10.1016/S0006-3207(01)00154-9

Oguz, T., Ducklow, H. W., Purcell, J. E., & Malanotte-Rizzoli, P. (2001). Modeling the responsae of top-down control exerted by gelatinous carnivores on the Black Sea pelagic food web. *Journal of Geophysical Research*, *106*, 4543–4564.

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2013). *Package 'vegan.'* Vegan: Community Ecology Package. R Package Version 2.0-10. https://doi.org/10.4135/9781412971874.n145

Oliva-Paterna, F. J., Ruiz-Navarro, A., Torralva, M., & Ferna ́ndez-Delgado, C. (2009). Biology of the endangered cyprinodontid Aphanius iberus in a saline wetland (SE Iberian Peninsula). *Italian Journal of Zoology*, *76*(3), 316–329. https://doi.org/10.1080/11250000802488159

Oliva-Paterna,F.J., García-Alonso, J., Cardozo, V., & Torralva, M. (2007). Field studies of ammonia excretion in Aphanius iberus (Pisces; Cyprinodontidae): Body size and habitat effects. *Journal of Applied Ichthyology*. https://doi.org/10.1111/j.1439-0426.2006.00791.x

Oliva-Paterna,F.J., Torralva, M., & Fernández-Delgado, C. (2006). Threatened fishes of the world: Aphanius iberus (Cuvier & Valenciennes, 1846) (Cyprinodontidae). *Environmental Biology of Fishes*, 75(3), 307-309. https://doi.org/10.1007/s10641-006-0016-2

Oltra, R., & Todolí, R. (2000). Reproduction of the endangered killifish Aphanius iberus at different salinities. *Environmental Biology of Fishes*. https://doi.org/10.1023/A:1007579527064

Paerl, H. W., & Otten, T. G. (2013). Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls. *Microbial Ecology*, *65*(4), 995–1010. https://doi.org/10.1007/s00248-012-0159-y

Parsons & Strickland. (1963). *Pigment analysis_strickland and parsons.pdf*.

Pearce, F., & Crivelli, A. (1994). Characteristics of Mediterranean Wet- lands. *MedWet Publications*, No.1. Tour du Valat.

Perennou, C., Beltrame, C., Guelmami, A., Tomas Vives, P., and C., & P. (2012). Existing areas and past changes of wetland extent in the Mediterranean region: an overview. *Ecologia Mediterranea*, 38, 53–66.

Persson, L., Bengtsson, J., Menge, B. A., & Power, M. E. (1996). Food Webs. Integration of Patterns and Dynamics. In *Global Change Biology*. https://doi.org/10.1353/aph.2013.0001

Peters & Wassenberg. (1983). The effect of body size on animal abundance. *Oecologia*, 60(1), 89-96.

Peters, R. H., & Downing, J. A. (1984). Empirical analysis of zooplankton filtering and feeding rates. *Limnology and Oceanography*, 29(4), 763-784. https://doi.org/10.4319/lo.1984.29.4.0763

Pielou, E. C. (1969). *An introduction to mathematical ecology*. Wiley-Interscience.

Pinheiro, J. C. (2021). *Package ' nlme ' Linear and Nonlinear Mixed Effects Models*.

Pinheiro, J. C., & Bates, D. M. (2000). Extending the Basic Linear. In *Mixed Effect Models in S & S-Plus*.

Pitt, K. A., Kingsford, M. J., Rissik, D., & Koop, K. (2007). Jellyfish modify the response of planktonic assemblages to nutrient pulses. *Marine Ecology Progress Series*, *351*, 1–13. https://doi.org/10.3354/meps07298

Platt, T., & Denman, K. (1977). Organisation in the pelagic ecosystem. *Helgoländer Wissenschaftliche*

Meeresuntersuchungen, *30*(1–4), 575–581. https://doi.org/10.1007/BF02207862

Plaut, I. (2000). Resting metabolic rate, critical swimming speed, and routine activity of the euryhaline cyprinodontid, Aphanius dispar, acclimated to a wide range of salinities. *Physiological and Biochemical Zoology*, 73(5), 590-596. https://doi.org/10.1086/317746

Polishchuk, L. V., & Blanchard, J. L. (2019). Uniting Discoveries of Abundance-Size Distributions from Soils and Seas. *Trends in Ecology and Evolution*, *34*(1), 2–5. https://doi.org/10.1016/j.tree.2018.10.007

Pomeranz, J. P. F., Warburton, H. J., & Harding, J. S. (2019). Anthropogenic mining alters macroinvertebrate size spectra in streams. *Freshwater Biology*, *64*(1), 81–92. https://doi.org/10.1111/fwb.13196

Post, D. M., & Takimoto, G. (2007). Proximate structural mechanisms for variation in food-chain length. *Oikos*, *116*(5), 775–782. https://doi.org/10.1111/j.2007.0030-1299.15552.x

Prado, P., Alcaraz, C., Jornet, L., Caiola, N., & Ibańẽz, C. (2017). Effects of enhanced hydrological connectivity on mediterranean salt marsh fish assemblages with emphasis on the endangered Spanish toothcarp(Aphanius iberus). *PeerJ*. https://doi.org/10.7717/peerj.3009

Prado, P., Vergara, C., Caiola, N., & Ibáñez, C. (2014). Influence of salinity regime on the food-web structure and feeding ecology of fish species from Mediterranean coastal lagoons. *Estuarine, Coastal and Shelf Science*, 139, 1-10. https://doi.org/10.1016/j.ecss.2013.12.013

Pyke, G. H. (2005). A review of the biology of Gambusia affinis and G. holbrooki. In *Reviews in Fish Biology and Fisheries*, 15(4), 339-365. https://doi.org/10.1007/s11160-006-6394-x

Quintana, XD. (2002). Estimation of water circulation in a Mediterranean salt marsh and its relationship with flooding causes. *Limnetica*, *21*(1–2), 25–35.

Quintana, XD., Arim, M., Badosa, A., Blanco, J. M., Boix, D., Brucet, S., Compte, J., Egozcue, J. J., de Eyto, E., Gaedke, U., Gascón, S., de Solá, L. G., Irvine, K., Jeppesen, E., Lauridsen, T. L., López-Flores, R., Mehner, T., Romo, S., & Søndergaard, M. (2014). Predation and competition effects on the size diversity of aquatic communities. *Aquatic Sciences*, *77*(1), 45–57. https://doi.org/10.1007/s00027-014-0368-1

Quintana, XD., Boix, D., Badosa, A., Brucet, S., Gascón, S., Gesti, J., & Trobajo, R. (2004). Limnología de los humedales costeros mediterráneos . El caso de Aiguamolls de l ′ Empordà . *Ecosistemas*, *13*(2), 30–36.

Quintana, XD., Boix, D., Badosa, A., Brucet, S., Compte, J., Gascon, S., López-Flores, R., Sala, J., & Moreno-Amich, R. (2006). Community structure in mediterranean shallow lentic ecosystems: Size-based vs. taxon-based approaches. *Limnetica*, vol 25, num. 1-2. p.303-320.

Quintana, XD., Brucet, S., Boix, D., López-Flores, R., Gascón, S., Badosa, A., Sala, J., Moreno-Amich, R., & Egozcue, J. J. (2008). A nonparametric method for the measurement of size diversity with emphasis on data standardization, 6(1), 75-86. *Limnology and Oceanography: Methods*. https://doi.org/10.4319/lom.2008.6.75

Quintana, XD., Comín, F. A., & Moreno-Amich, R. (2002). Biomass-size spectra in aquatic communities in shallow fluctuating Mediterranean salt marshes (Empordà wetlands, NE Spain). *Journal of Plankton Research*, 24(11), 1149-1161. https://doi.org/10.1093/plankt/24.11.1149

Quintana, XD., Egozcue, J. J., Martínez-Abella, O., López-Flores, R., Gascón, S., Brucet, S., & Boix, D. (2016). Update: A non-parametric method for the measurement of size diversity, with emphasis on data standardization. The measurement of the size evenness. *Limnology and Oceanography: Methods*, *14*(6), 408–413. https://doi.org/10.1002/lom3.10099

Quintana, XD., Moreno-Amich, R., & Comín, F. A. (1998). Nutrient and plankton dynamics in a Mediterranean salt marsh dominated by incidents of flooding. Part 1: Differential confinement of nutrients. *Journal of Plankton Research*, 20(11), 2089-2107. https://doi.org/10.1093/plankt/20.11.2089

Quintana, X.D., Arim, M., Badosa, A., Blanco, J. M., Boix, D., Brucet, S., Compte, J., Egozcue, J. J., de Eyto, E., Gaedke, U., Gascón, S., de Solá, L. G., Irvine, K., Jeppesen, E., Lauridsen, T. L., López-Flores, R., Mehner, T., Romo, S., & Søndergaard, M. (2015). Predation and competition effects on the size diversity of aquatic communities. *Aquatic Sciences*, *77*(1), 45–57. https://doi.org/10.1007/s00027-014-0368-1

Quintana, X.D., Cañedo-Argüelles, M., Nebra, A., Gascón, S., Rieradevall, M., Caiola, N., Sala, J., Ibàñez, C., Sánchez-Millaruelo, N., & Boix, D. (2015). *New Tools to Analyse the Ecological Status of Mediterranean Wetlands and Shallow Lakes* (pp. 171–199). https://doi.org/10.1007/698_2015_391

Rhazi, M., Grillas, P., Charpentier, A., & Médail, F. (2004). Experimental management of Mediterranean temporary pools for conservation of the rare quillwort Isoetes setacea. *Biological Conservation*, *118*(5), 675–684. https://doi.org/10.1016/j.biocon.2003.10.016

Rincón, P. A., Correas, A. M., Morcillo, F., Risueño, P., & Lobón-Cerviá, J. (2002). Interaction between the introduced eastern mosquitofish and two autochthonous Spanish toothcarps. *Journal of Fish Biology*, 61(6), 1560-1585. https://doi.org/10.1006/jfbi.2002.2175

Rodriguez, M. A., & Magnan, P. (1993). Community structure of lacustrine macrobenthos: do taxon-based and size-based approaches yield similar insights? *Canadian Journal of Fisheries and Aquatic Sciences*, *50*(4), 800–

815. https://doi.org/10.1139/f93-093

Romo, S., & Villena, M. J. (2005). Phytoplankton strategies and diversity under different nutrient levels and planktivorous fish densities in a shallow Mediterranean lake. *Journal of Plankton Research*, 27(12), 1273-1286. https://doi.org/10.1093/plankt/fbi093

Ruhí, A., Boix, D., Sala, J., Gascón, S., & Quintana, X. D. (2009). Spatial and temporal patterns of pioneer macrofauna in recently created ponds: Taxonomic and functional approaches. *Hydrobiologia*, *634*(1), 137–151. https://doi.org/10.1007/s10750-009-9896-4

Ruhí, A., Chappuis, E., Escoriza, D., Jover, M., Sala, J., Boix, D., Gascón, S., & Gacia, E. (2014). Environmental filtering determines community patterns in temporary wetlands: A multi-taxon approach. *Hydrobiologia*, *723*(1), 25–39. https://doi.org/10.1007/s10750-013-1514-9

Ruttner-Kolisko, A. (1977). Suggestions for biomass calculations of planktonic rotifers. *Ergeb. Limnol*, 21, 71-76.

Sahuquillo, M., Miracle, M. R., Morata, S. M., & Vicente, E. (2012). Nutrient dynamics in water and sediment of Mediterranean ponds across a wide hydroperiod gradient. *Limnologica*, *42*(4), 282–290. https://doi.org/10.1016/j.limno.2012.08.007

Sala, J., Gascón, S., Boix, D., Gesti, J., & Quintana, X. D. (2004). Proposal of a rapid methodology to assess the conservation status of Mediterranean wetlands and its application in Catalunya (NE Iberian Peninsula). *Archives Des Sciences*, 57, 141-152I.

Salvadó, V., Quintana, X. D., & Hidalgo, M. (2006). Monitoring of nutrients, pesticides, and metals in waters, sediments, and fish of a wetland. *Archives of Environmental Contamination and Toxicology*, *51*(3), 377–386. https://doi.org/10.1007/s00244-005-0107-7

Schneider, D. W. (1999). Snowmelt Ponds in Wisconsin. *Invertebrates in Freshwater Wetlands of North America: Ecology and Management*, 299–318.

Schneider, D. W., & Frost, T. M. (1996). Habitat duration and community structure in temporary ponds. *Journal of the North American Benthological Society*, *15*(1), 64–86. https://doi.org/10.2307/1467433

Scott & Murdoch. (1983). Selective predation by the backswimmer, Notonecta. *Limnol. Oceanogr.*, *28*(2), 352–366.

Sen Gupta, R., & Koroleff, F. (1973). A quantitative study of nutrient fractions and a stoichiometric model of the Baltic. *Estuarine and Coastal Marine Science*, 1(4), 335-360. https://doi.org/10.1016/0302-3524(73)90025-X

Sgarzi, S., Badosa, A., Leiva-Presa, À., Benejam, L., López-Flores, R., & Brucet, S. (2019). Plankton taxonomic and size diversity of Mediterranean brackish ponds in spring: Influence of abiotic and biotic factors. *Water (Switzerland)*, 11(1), 106. https://doi.org/10.3390/w11010106

Sheldon, R. W., Prakash, A., & Sutcliffe, H. (1972). The size distribution of particles in the Ocean 1.*Limnology and Oceanography 17(3), 327-340*

Sherr, E., & Sherr, B. (1988). Role of microbes in pelagic food webs: a revised concept. *Limnology and Oceanography*, *33*(5), 1225–1227.

Shin, Y. J., Rochet, M. J., Jennings, S., Field, J. G., & Gislason, H. (2005). Using size-based indicators to evaluate the ecosystem effects of fishing. *ICES Journal of Marine Science*, *62*(3), 384–396. https://doi.org/10.1016/j.icesjms.2005.01.004

Sim, L. L., Davis, J. A., Strehlow, K., McGuire, M., Trayler, K. M., Wild, S., Papas, P. J., & O'Connor, J. (2013). The influence of changing hydroregime on the invertebrate communities of temporary seasonal wetlands. *Freshwater Science*, *32*(1), 327–342. https://doi.org/10.1899/12-024.1

Smock, L. (1980). Relationships between body size and biomass of aquatic insects. *Freshwater Biology*, *10*, 375–383.

Sommer, F., Ståhlman, M., Ilkayeva, O., Arnemo, J. M., Kindberg, J., Josefsson, J., Newgard, C. B., Fröbert, O., & Bäckhed, F. (2016). The Gut Microbiota Modulates Energy Metabolism in the Hibernating Brown Bear Ursus arctos. *Cell Reports*, *14*(7), 1655–1661. https://doi.org/10.1016/j.celrep.2016.01.026

Søndergaard, M., Jeppesen, E., & Jensen, J. P. (2005). Pond or lake: Does it make any difference? *Archiv Fur Hydrobiologie*, 162(2), 143-165. https://doi.org/10.1127/0003-9136/2005/0162-0143

Søndergaard, M., Kristensen, P., & Jeppesen, E. (1992). Phosphorus release from resuspended sediment in the shallow and wind-exposed Lake Arresø, Denmark. *Hydrobiologia*, *228*(1), 91–99. https://doi.org/10.1007/BF00006480

Sosa-López, A., Mouillot, D., Ramos-Miranda, J., Flores-Hernandez, D., & Chi, T. Do. (2007). Fish species richness decreases with salinity in tropical coastal lagoons. *Journal of Biogeography*, *34*(1), 52–61. https://doi.org/10.1111/j.1365-2699.2006.01588.x

Specchiulli, A., Focardi, S., Renzi, M., Scirocco, T., Cilenti, L., Breber, P., & Bastianoni, S. (2008). Environmental heterogeneity patterns and assessment of trophic levels in two Mediterranean lagoons: Orbetello and Varano, Italy. *Science of the Total Environment*, *402*(2–3), 285–298. https://doi.org/10.1016/j.scitotenv.2008.04.052

Spencer, M., Blaustein, L., Schwartz, S. ., & Cohen, J. (1999). Species richness and the proportion of predatory animal species in temporary freshwater pools: relationships with habitat size and permanence. *Ecol. Lett.*, *2*, 157–166.

Sprules, W. G., & Barth, L. E. (2016). Surfing the biomass size spectrum: Some remarks on history, theory, and application. *Canadian Journal of Fisheries and Aquatic Sciences*, *73*(4), 477–495. https://doi.org/10.1139/cjfas-2015-0115

Sprules, W. G., & Munawar, M. (1986). Plankton size spectra in relation to ecosystem productivity, size, and perturbation. *Canadian Journal of Fisheries and Aquatic Sciences*, *43*(9), 1789–1794. https://doi.org/10.1139/f86-222

Strayer, D. L. (1991). Perspectives on the Size Structure of Lacustrine Zoobenthos, Its Causes, and Its Consequences. *Journal of the North American Benthological Society*, *10*(2), 210–221. https://doi.org/10.2307/1467579

Sun, J., & Liu, D. (2003). Geometric models for calculating cell biovolume and surface area for phytoplankton. *Journal of Plankton Research*, *25*(11), 1331–1346. https://doi.org/10.1093/plankt/fbg096

Tittel, J., Zippel, B., Geller, W., & Seeger, J. (1998). Relationships between plankton community structure and plankton size distribution in lakes of northern Germany. *Limnology and Oceanography*, *43*(6), 1119–1132. https://doi.org/10.4319/lo.1998.43.6.1119

Traina, J. A., & von Ende, C. N. (1992). Estimation of larval dry weight of Chaoborus americanus. *Hydrobiologia*, *228*(3), 219–223. https://doi.org/10.1007/BF00006588

Treblico, R., Baum, J. K., Salomon, A. K., & Dulvy, N. K. (2013). Ecosystem ecology: Size-based constraints on the pyramids of life. *Trends in Ecology and Evolution*, *28*(7), 423–431. https://doi.org/10.1016/j.tree.2013.03.008

Tryjanowski, P., Sparks, T., Rybacki, M., & Berger, L. (2006). Is body size of the water frog *Rana esculenta* complex responding to climate change? *Naturwissenschaften*, *93*(3), 110–113. https://doi.org/10.1007/s00114-006-0085-2

Van Den Broeck, M., Waterkeyn, A., Rhazi, L., Grillas, P., & Brendonck, L. (2015). Assessing the ecological integrity of endorheic wetlands, with focus on Mediterranean temporary ponds. *Ecological Indicators*, *54*, 1–11. https://doi.org/10.1016/j.ecolind.2015.02.016

Vanni, M. J. (1986). Fish predation and zooplankton demography: indirect effects. *Ecology*, *67(2)*, 337–354.

Vargas, M. J. (1993). *Interaccion entre Aphanius iberus y Gambusia holbrooki en el delta del Ebro: sus ciclos biologicos y ecologıas troficas.* (Doctoral dissertation, Universitat de Barcelona).

Vargas, M. J., & De Sostoa, A. (1997). Life-history pattern of the Iberian toothcarp Aphanius iberus (Pisces, Cyprinodontidae) from a Mediterranean estuary, the Ebro Delta (Spain). *Netherlands Journal of Zoology*, 12(44), 1523. https://doi.org/10.1163/156854297x00157

Vila-Gispert, A., & Moreno-Amich, R. (2001). Mass-length relationship of Mediterranean barbel as an indicator of environmental status in South-west European stream ecosystems. *Journal of Fish Biology*, *59*(4), 824–832. https://doi.org/10.1006/jfbi.2001.1697

Vilà, M., & García-Berthou, E. (2010). Monitoring biological invasions in freshwater habitats. In *Conservation Monitoring in Freshwater Habitats A Practical Guide and Case Studies*. pp 91-100. https://doi.org/10.1007/978-1-4020-9278-7_10

Villena, M. J., & Romo, S. (2003). Phytoplankton changes in a shallow Mediterranean lake (Albufera of Valencia, Spain) after sewage diversion. *Hydrobiologia*, *506–509*, 281–287. https://doi.org/10.1023/B:HYDR.0000008565.23626.aa

Waterkeyn, A., Grillas, P., Vanschoenwinkel, B., & Brendonck, L. (2008). Invertebrate community patterns in Mediterranean temporary wetlands along hydroperiod and salinity gradients. *Freshwater Biology*, *53*(9), 1808–1822. https://doi.org/10.1111/j.1365-2427.2008.02005.x

Webster, K. E., & Peters, R. H. (1978). Some size-dependent inhibitions of larger cladoceran filterers in filamentous suspensions. *Limnology and Oceanography*, *23*(6), 1238–1245. https://doi.org/10.4319/lo.1978.23.6.1238

Wellborn, G. A., Skelly, D. K., & Werner, E. E. (1996). Mechanisms creating community structure across a freshwater habitat gradient. *Annual Review of Ecology and Systematics*, *27*(January), 337–363. https://doi.org/10.1146/annurev.ecolsys.27.1.337

White, E. P. (2008). On estimating the exponent of power-law frequency distributions. *Ecology*, *89*(4), 905–912.

Wickham, H. (2016). ggplot2 Elegant Graphics for Data Analysis (Use R!). In *Springer*.

Williams, D.D. (1997). Temporary ponds and their invertebrate communities. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *7*(2), 105–117. https://doi.org/10.1002/(SICI)1099-0755(199706)7:2<105::AID-AQC222>3.0.CO;2-K

Williams, D.D. (2006). The biology of temporary waters. In *Oxford University Press, Oxford*.

Williams, P., Whitfield, M., Biggs, J., Bray, S., Fox, G., Nicolet, P., & Sear, D. (2004). Comparative biodiversity of rivers, streams, ditches and ponds in an agricultural landscape in Southern England. *Biological Conservation*, *115*(2), 329–341. https://doi.org/10.1016/S0006-3207(03)00153-8

Woodward, G., Ebenman, B., Emmerson, M., Montoya, J. M., Olesen, J. M., Valido, A., & Warren, P. H. (2005). Body size in ecological networks. *Trends in Ecology and Evolution*, *20*(7), 402–409. https://doi.org/10.1016/j.tree.2005.04.005

Woodward, G., & Hildrew, A. G. (2002). Body-size determinants of niche overlap and intraguild predation within a complex food web. *Journal of Animal Ecology*, *71*(6), 1063–1074. https://doi.org/10.1046/j.1365-2656.2002.00669.x

Wootton, R. J. (1989). Ecology of Teleost Fishes. In *Ecology of Teleost Fishes*. https://doi.org/10.1007/978-94-009-0829-1

Worm, B., Barbier, E. B., Beaumont, N., Duffy, E., Folke, C., Halpern, B. S., Jackson, J. B. C., Lotze, H. K., Micheli, F., Palumbi, S. R., Sala, E., Selkoe, K. A., Stachowicz, J. J., & Watson, R. (2006). Impacts of biodiversity loss on ocean ecosystem services. *science*, *314*(5800), 787-790.

Ye, L., Chang, C. Y., García-Comas, C., Gong, G. C., & Hsieh, C. H. (2013). Increasing zooplankton size diversity enhances the strength of top-down control on phytoplankton through diet niche partitioning. *Journal of Animal Ecology*, 82(5), 1052-1061. https://doi.org/10.1111/1365-2656.12067

Yildirim, Ö., & Karacuha, A. (2008). Effect of salinity on growth performance and survival rate of *Aphanius chantrei* (Galliard, 1895). *Journal of Applied Ichthyology*, 24(3), 345-347. https://doi.org/10.1111/j.1439-0426.2007.01053.x

Yvon-Durocher, G., & Allen, A. P. (2012). Linking community size structure and ecosystem functioning using metabolic theory. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1605), 2998–3007. https://doi.org/10.1098/rstb.2012.0246

Yvon-Durocher, G., Montoya, J. M., Trimmer, M., & Woodward, G. (2011). Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems. *Global Change Biology*, *17*(4), 1681–1694. https://doi.org/10.1111/j.1365-2486.2010.02321.x

Zacharias, I., Parasidoy, A., Bergmeier, E., Kehayias, G., Dimitriou, E., & Dimopoulos, P. (2008). A "DPSIR" model for Mediterranean temporary ponds: European, national and local scale comparisons. *Annales de Limnologie*, *44*(4), 253–266. https://doi.org/10.1051/limn:2008009

Zacharias, & Zamparas, M. (2010). Mediterranean temporary ponds. A disappearing ecosystem. *Biodiversity and Conservation*, *19*(14), 3827–3834. https://doi.org/10.1007/s10531-010-9933-7

Zedler, J. B., & Kercher, S. (2005). Wetland resources: Status, trends, ecosystem services, and restorability. *Annual Review of Environment and Resources*, *30*, 39–74. https://doi.org/10.1146/annurev.energy.30.050504.144248

Zhang, J., Xie, P., Tao, M., Guo, L., Chen, J., Li, L., XueZhen Zhang, & Zhang, L. (2013). The Impact of Fish Predation and Cyanobacteria on Zooplankton Size Structure in 96 Subtropical Lakes. *PLoS ONE*, *8*(10), 1–15. https://doi.org/10.1371/journal.pone.0076378

Zimmer, K. D., Hanson, M. A., Butler, M. G., & Duffy, W. G. (2001). Size distribution of aquatic invertebrates in two prairie wetlands, with and without fish, with implications for community production. *Freshwater Biology*, *46*(10), 1373–1386. https://doi.org/10.1046/j.1365-2427.2001.00759.x

11. SUPPORTING INFORMATION

Photo S1. From the left, photography of one of the studied permanent brackish ponds (Bassa Nova) in 'El Montgrí, Illes Medes i el Baix Ter' Natural Park and one of the studied freshwater temporary freshwater ponds (Cardonera) in Albera. (Author: Serena Sgarzi).

Photo S2. The image shows the sampling of macroinvertebrates in temporary ponds (Authors: Carmen García-Comas).

Photo S3. Plastic minnow traps set up in one of the studied Mediterranean brackish ponds. (Author: Serena Sgarzi).

Photo S4. Fyke net set up in one of the studied Mediterranean brackish ponds. (Author: Serena Sgarzi).

Photo S5. The photo on the left, shows individuals of *Aphanius iberus* (female on the left, male on the right) during a body length measurement in Mediterranean brackish ponds. The individuals were quickly returned into the pond after measurement. The photo on the right shows macroinvertebrates and microcrustacean sampled in one of the Mediterranean freshwater ponds studied in spring season. (Author: Serena Sgarzi).

Table S1. Conductivity of the brackish studied ponds, along with the number of traps used for each pond.

POND NAME	ID	CONDUCTIVITY (mS·cm^{-1})	NUMBER OF BOTTLES	NUMBER OF FYKE NETS	NUMBER OF GILNETS
Bassa del fartet	BF	10.07	36	4	0
La Rubina	RUB	12.48	18	2	0
Bassa nova	BN	26.37	50	4	1
Camping out	CO	31.06	38	3	0
Frare Ramon	FR	40.62	80	10	1
Camping nord	CN	40.97	74	6	2
Camping sud	CS	43.03	80	5	2
Túries	TU	45.85	96	12	0
Connectada	CON	53.69	42	4	0
Bassa del Pi	BPI	54.53	38	2	0
Bassa de la llúdriga	LLU	59.27	58	6	1
Bassa de l'anguila	AN	66.89	54	6	1
Fangassos	FAN	69.10	16	2	0

Table S2. Results of the Full models of GLMs (N=13) of Chapter 1, showing the predictor variables that affect size diversity and taxonomic diversity of phytoplankton and zooplankton assemblages. Both full models and best models are presented. For each one, intercept (estimate and standard error, S.E.), Beta coefficients (standardized), t-value, significance (*p-value*) and R square of the model are shown. Phyt.biom.:TN is the ratio of Phytoplankton biomass: Total Nitrogen.

Response Variable	**Model**	**AIC**	**Predictors**	**Estimate**	**S.E.**	**Beta Coefficients**	***t*-Value**	***p*-Value**	**R-Square**
Zooplankton									
Size diversity	Full	32.00	Conductivity	<0.01	0.01	0.22	0.73	0.49	0.08
			Log Pond Area	0.43	0.26	0.50	1.66	0.14	
			Fish size diversity	−0.74	0.58	−0.37	−1.28	0.24	
			Phytoplankton size diversity	−0.12	0.32	−0.11	−0.37	0.72	
Species diversity	Full	12.20	Conductivity	<0.01	0.01	−0.15	−0.55	0.60	0.19
			Log Pond Area	0.27	0.14	0.60	1.89	0.09	
			Fish Shannon diversity	<0.01	0.69	<0.01	<0.01	0.99	
			Phytoplankton Shannon diversity	0.11	0.21	0.17	0.52	0.62	
	Best	6.90	Log Pond Area	0.29	0.10	0.65	2.86	0.02	0.42
Phytoplankton									
Size diversity	Full	26.80	Conductivity	0.01	0.01	0.41	1.24	0.25	0.28
			Log Pond Area	0.18	0.23	0.21	0.75	0.48	
			Log Pond Depth	−0.63	0.72	−0.27	−0.88	0.41	
			Zooplankton size diversity	−0.22	0.28	−0.24	−0.79	0.45	
			Phytoplankton biomass: TN	−2.96	2.00	−0.49	−1.48	0.18	
	Best	23.90	Conductivity	0.02	0.01	0.48	2.04	0.06	0.47
			Phytoplankton biomass: TN	−3.71	1.42	−0.62	−2.62	0.02	
Species diversity	Full	14.00	Conductivity	0.01	0.01	0.15	0.86	0.42	0.65
			Log Pond Area	0.21	0.16	0.34	1.31	0.23	
			Log Pond Depth	−1.15	0.44	−0.61	−2.62	0.03	
			Zooplankton Shannon diversity	0.17	0.28	0.06	0.63	0.55	
			Phytoplankton biomass: TN	−1.01	1.14	−0.18	−0.88	0.41	
	Best	9.34	Log Pon Area	0.32	0.11	0.46	2.96	0.01	0.76
			Log Pond Depth	−1.44	0.31	−0.73	−4.73	<0.01	

Table S3. Results of the MLMs (N = 49) of Chapter 2, showing the predictor variables that affect size-related variables and abundance (CPUE) of *Aphanius iberus*. Both Full models and best models are presented. For each one, intercept (estimate and standard error, S.E.), Beta coefficients (standardized), t-value, significance (p-value), and df are shown.

Response variable	AIC	Predictor	Estimate	S.E.	Beta coefficients	t-value	p-value	df
***Aphanius iberus* MAXIMUM length**								
Full & Best model	793.44	Conductivity	-19.49	4.69	-0.47	-4.15	<0.01	5
		Log Total Nitrogen	2325.09	564.20	0.45	4.12	<0.01	5
		QAELS index	3894.26	690.27	0.59	5.64	<0.01	5
		Zooplankton biomass	-0.22	0.05	-0.69	-4.51	<0.01	5
***Aphanius iberus* MEAN length**								
Full model	289.55	Conductivity	-2.631	0.713	-0.496	-3.688	0.01	5
		Zooplankton biomass	-0.002	0.001	-0.639	-3.525	0.02	5
		Log Total Nitrogen	0.675	0.640	0.093	1.053	0.34	5
		QAELS index	3.237	0.679	0.601	4.765	0.01	5
Best model	288.78	Conductivity	-0.15	0.05	-0.42	-3.35	0.02	6
		QAELS index	31.94	6.76	0.55	4.73	<0.01	6
		Zooplankton biomass	-0.01	-0.01	-0.59	-4.01	0.01	6
***Aphanius iberus* length RANGE**								
Full model	368.46	Conductivity	-0.612	1.762	0.022	-0.351	0.740	5
		Log Pond Mean Depth	3.930	1.876	0.490	2.095	0.09	5
		QAELS index	1.192	1.631	0.059	0.731	0.50	5
		Log Total Nitrogen	4.559	1.398	0.483	3.262	0.02	5
Best model	365.06	Log Pond Mean Depth	14.45	5.36	0.42	2.70	0.03	7
		Log Total Nitrogen	36.88	8.93	0.48	4.13	<0.01	7
***Aphanius iberus* SIZE DIVERSITY**								
Full model	-32.26	Conductivity	0.052	0.026	0.299	2.016	0.10	5
		Log Pond Mean Depth	-0.011	0.024	-0.061	-0.435	0.68	5
		QAELS index	0.01	0.025	0.041	0.282	0.79	5
		Log Total Nitrogen	0.044	0.023	0.254	1.943	0.11	5
Best model	-36.02	Conductivity	<0.01	<0.01	0.29	2.21	0.06	7
		Log Total Nitrogen	0.93	0.37	0.30	2.51	0.04	7
***Aphanius iberus* capture per effort unit (CPUE)**								
Full model	108.98	Log Pond Mean Depth	0.512	0.292	0.622	1.752	0.14	5
		QAELS index	0.080	0.238	-0.019	0.335	0.75	5
		Log Total Nitrogen	0.377	0.198	0.429	1.906	0.12	5
		Conductivity	-0.060	0.262	0.086	-0.230	0.83	5
Best model	105.17	Log Pond Mean Depth	1.46	0.57	0.55	2.56	0.04	7
		Log Total Nitrogen	2.15	0.97	0.36	2.23	0.06	7

Table S4. Results of the Linear Mixed Models (N=25) of Chapter 3, showing the predictor variables that affect the slopes and the intercepts of the ecological (phytoplankton, zooplankton) and physiological (phytoplankton and zooplankton together) size spectra (SS). Only the most parsimonious significant models are presented. For each one, estimate, standard error (S.E.), Beta coefficients (standardized), *t*-value, significance (*p*-value) and degrees of freedom (df) are shown. For each model, R^2 (the proportion of variance expressed by predictors) are shown, together with the AIC (Akaike's Information Criterion). Significant predictor variables were PCA1 (scores for the PCA axis 1) and DEPTH (mean water column depth). Both Full and Best models are shown.

Response variable	Predictor variable	Estimate	S.E.	Beta coefficients	*t* - Value	*p* - Value	df	R^2	AIC
Zooplankton SS slope	PCA1	0.004	0.04	0.03	0.11	0.91	14	0.09	16,5
	Depth	-0.05	0.04	-0.34	-1.30	0.21	14		
	Macroinvertebrates biomass	0.02	0.04	0.16	0.62	0.55	14		
Zooplankton SS intercept	PCA1	0.48	0.17	0.32	2.74	0.02	14	0.41	67.1
	Depth	-0.43	0.17	-0.32	-2.59	0.02	14		
	Macroinvertebrates biomass	-0.17	0.17	-0.22	-1.02	0.32	14		
Phytoplankton SS slope	PCA1	0.14	0.11	0.35	1.33	0.20	15	0.10	47.0
	Depth	-0.03	0.11	-0.06	-0.22	0.82	15		
	Zooplankton intercept	-0.03	0.12	-0.07	-0.25	0.81	15		
Phytoplankton SS intercept	PCA1	0.52	0.23	0.53	2.23	0.04	15	0.23	74.0
	Depth	-0.04	0.26	-0.04	-0.17	0.87	15		
	Zooplankton intercept	-0.11	0.27	-0.11	-0.42	0.68	15		
Phytoplankton + Zooplankton SS slope	PCA1	0.03	0.05	-0.09	0.61	0.55	15	0.24	23.1
	Depth	-0.14	0.05	-0.47	-2.85	0.01	15		
	Macroinvertebrates biomass	0.01	0.05	<0.01	0.21	0.83	15		
Phytoplankton + Zooplankton SS intercept	PCA1	0.79	0.47	<0.01	1.67	0.12	15	0.35	101.9
	Depth	-1.87	0.49	-0.45	-3.82	<0.01	15		
	Macroinvertebrates biomass	-0.54	0.49	-0.21	-1.09	0.29	15		

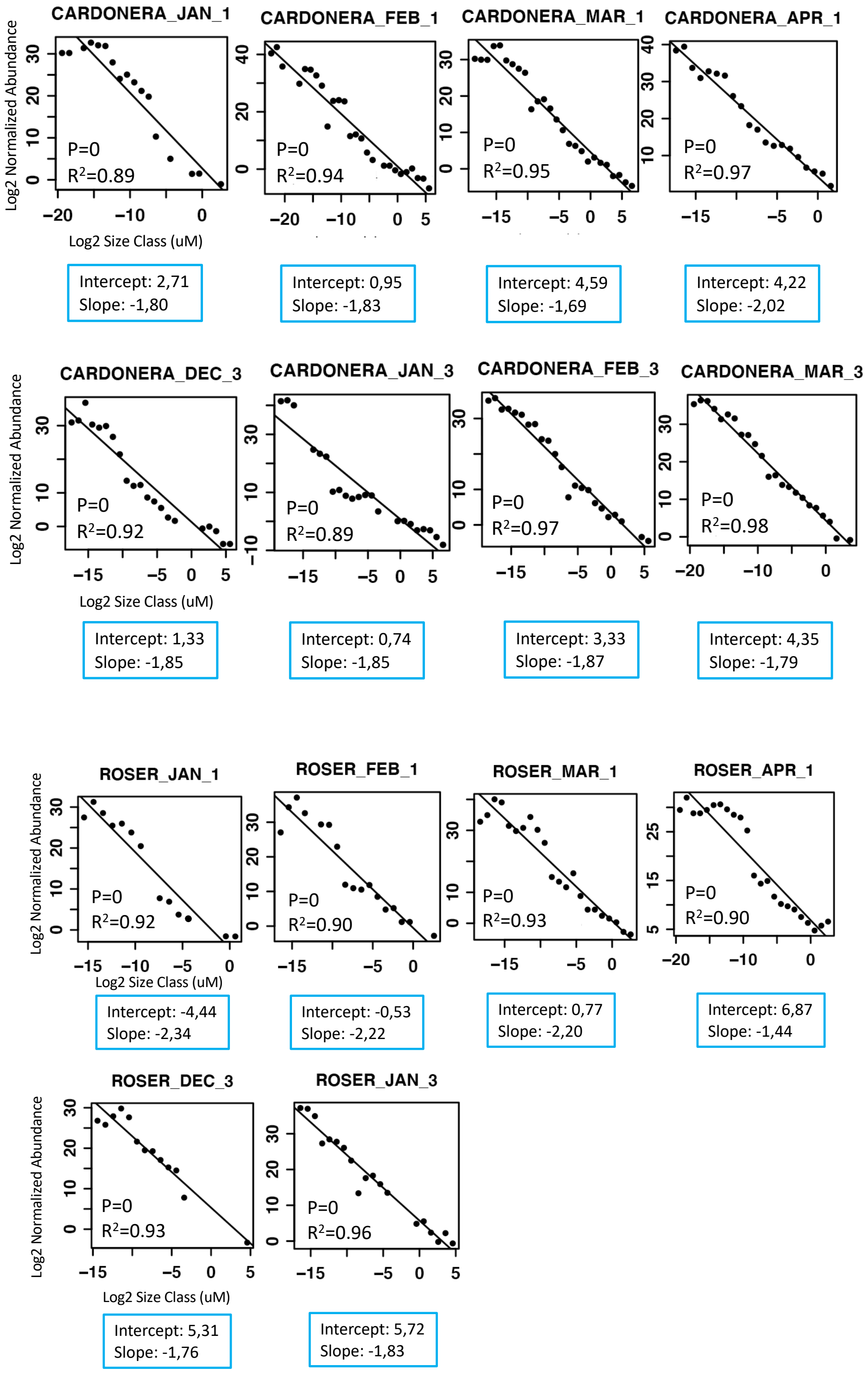
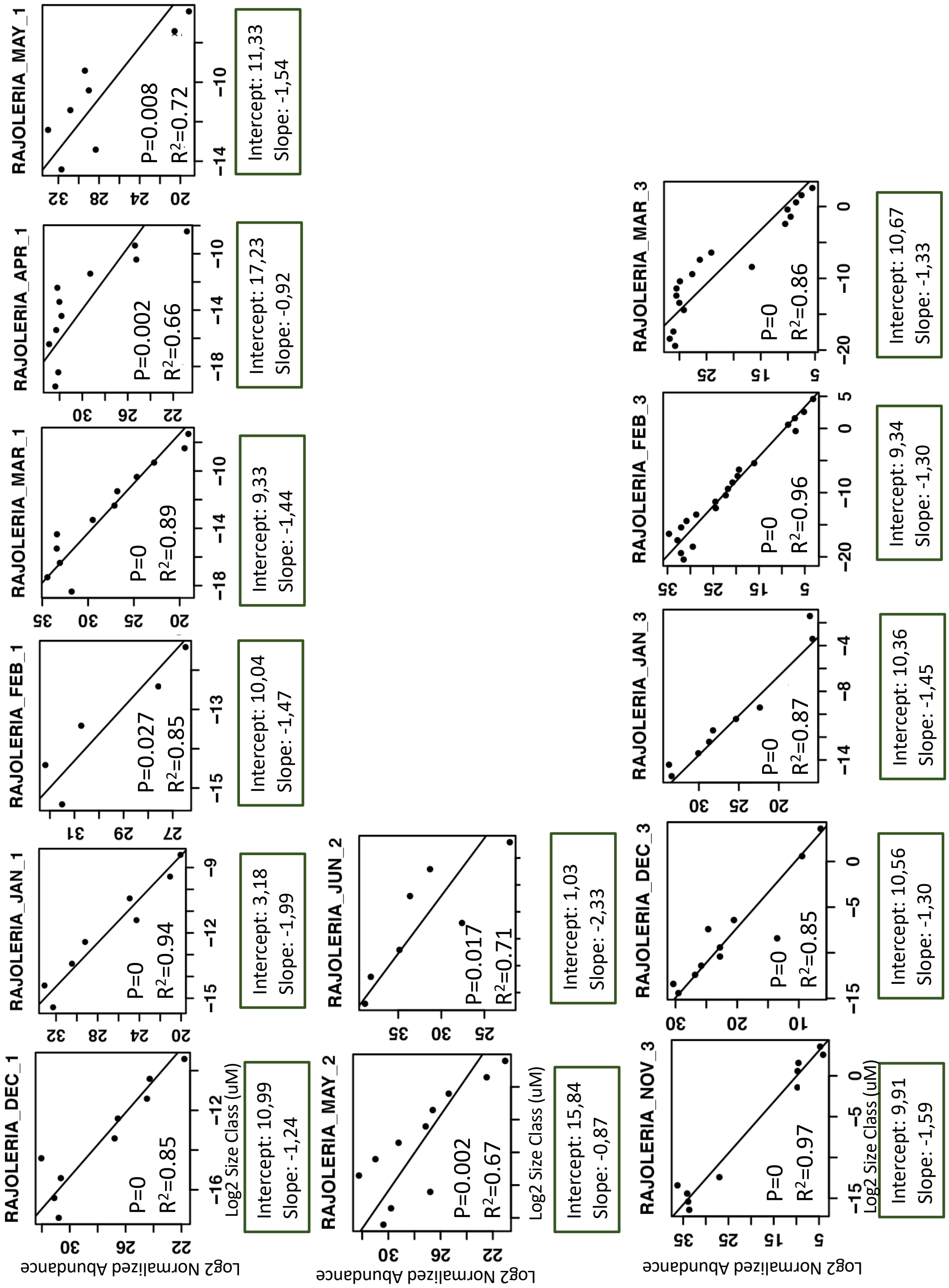


Figure S1. Physiological size spectra of phytoplankton + zooplankton in Cardonera and Prats del Roser, for each month sampled during the three hydroperiods. On the bottom of each size spectrum, intercepts and slopes values are shown. The pond name is indicated on the top of each spectrum, together with the month. The number following the month indicates the hydroperiod (1,2,3).


RAJOLERIA_DEC_1
P=0
R²=0.85
Intercept: 10,99
Slope: -1,24
RAJOLERIA_JAN_1
P=0
R²=0.94
Intercept: 3,18
Slope: -1,99
RAJOLERIA_FEB_1
P=0.027
R²=0.85
Intercept: 10,04
Slope: -1,47
RAJOLERIA_MAR_1
P=0
R²=0.89
Intercept: 9,33
Slope: -1,44
RAJOLERIA_APR_1
P=0.002
R²=0.66
Intercept: 17,23
Slope: -0,92
RAJOLERIA_MAY_1
P=0.008
R²=0.72
Intercept: 11,33
Slope: -1,54
RAJOLERIA_MAY_2
P=0.002
R²=0.67
Intercept: 15,84
Slope: -0,87
RAJOLERIA_JUN_2
P=0.017
R²=0.71
Intercept: 1,03
Slope: -2,33
RAJOLERIA_NOV_3
P=0
R²=0.97
Intercept: 9,91
Slope: -1,59
RAJOLERIA_DEC_3
P=0
R²=0.85
Intercept: 10,56
Slope: -1,30
RAJOLERIA_JAN_3
P=0
R²=0.87
Intercept: 10,36
Slope: -1,45
RAJOLERIA_FEB_3
P=0
R²=0.96
Intercept: 9,34
Slope: -1,30
RAJOLERIA_MAR_3
P=0
R²=0.86
Intercept: 10,67
Slope: -1,33
Log2 Normalized Abundance
Log2 Size Class (uM)

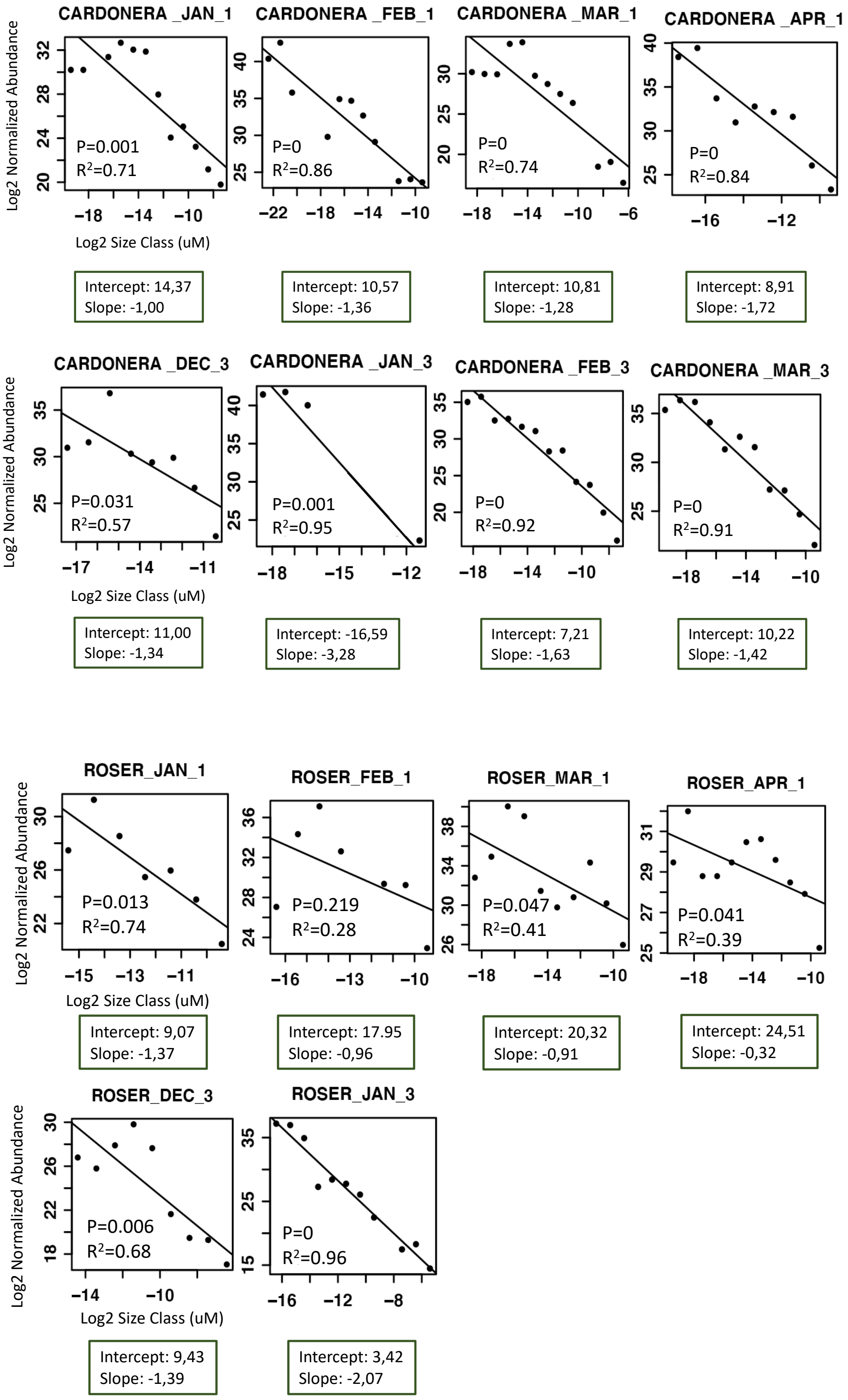
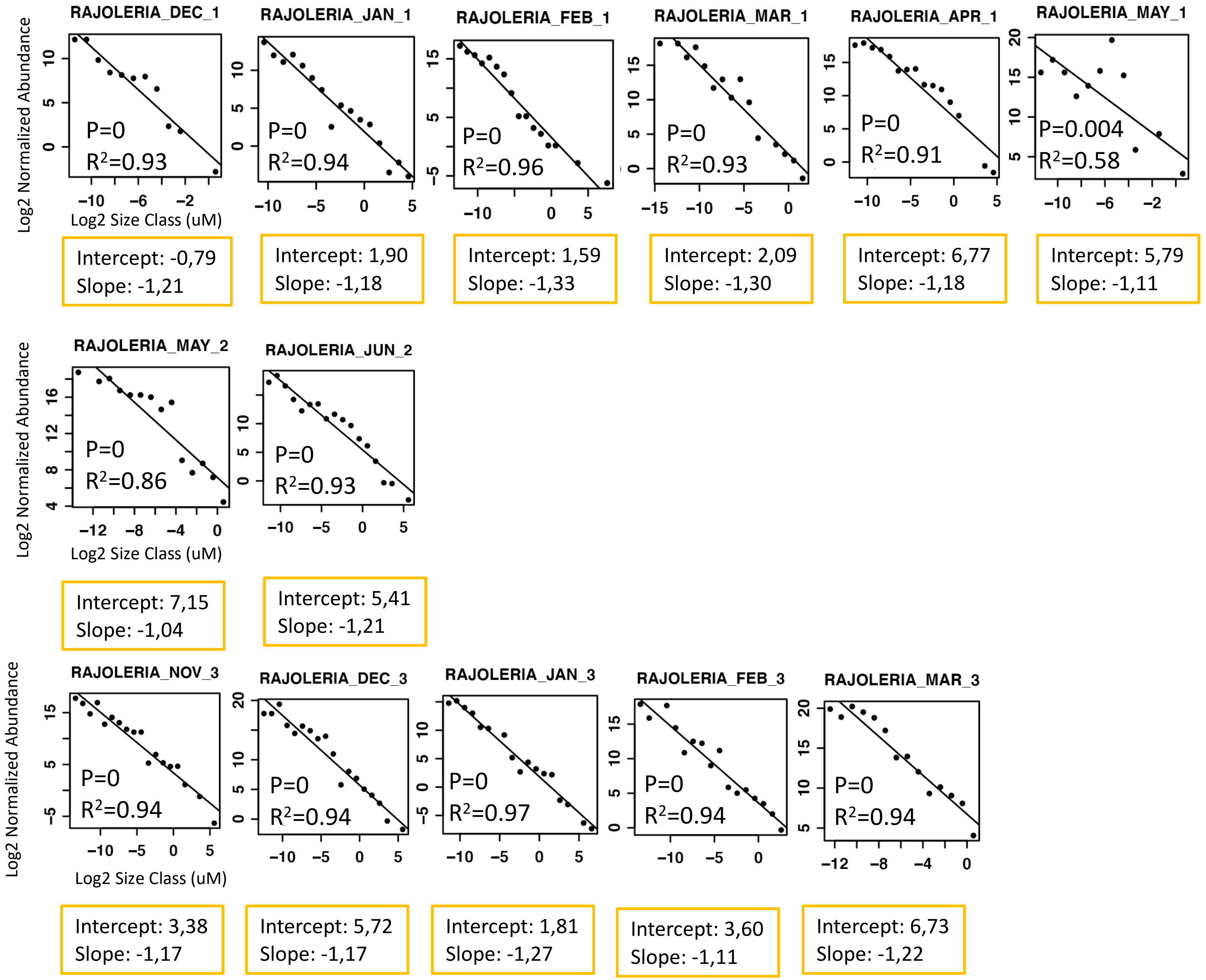


Figure S2. Ecological size spectra of phytoplankton in each pond, for each month sampled during the three hydroperiods. On the bottom of each size spectrum, intercepts and slopes values are shown. The pond name is indicated on the top of each spectrum, together with the month. The number following the month indicates the hydroperiod (1,2,3).


RAJOLERIA_DEC_1
P=0
R²=0.93
Intercept: -0,79
Slope: -1,21
RAJOLERIA_JAN_1
P=0
R²=0.94
Intercept: 1,90
Slope: -1,18
RAJOLERIA_FEB_1
P=0
R²=0.96
Intercept: 1,59
Slope: -1,33
RAJOLERIA_MAR_1
P=0
R²=0.93
Intercept: 2,09
Slope: -1,30
RAJOLERIA_APR_1
P=0
R²=0.91
Intercept: 6,77
Slope: -1,18
RAJOLERIA_MAY_1
P=0.004
R²=0.58
Intercept: 5,79
Slope: -1,11
RAJOLERIA_MAY_2
P=0
R²=0.86
Intercept: 7,15
Slope: -1,04
RAJOLERIA_JUN_2
P=0
R²=0.93
Intercept: 5,41
Slope: -1,21
RAJOLERIA_NOV_3
P=0
R²=0.94
Intercept: 3,38
Slope: -1,17
RAJOLERIA_DEC_3
P=0
R²=0.94
Intercept: 5,72
Slope: -1,17
RAJOLERIA_JAN_3
P=0
R²=0.97
Intercept: 1,81
Slope: -1,27
RAJOLERIA_FEB_3
P=0
R²=0.94
Intercept: 3,60
Slope: -1,11
RAJOLERIA_MAR_3
P=0
R²=0.94
Intercept: 6,73
Slope: -1,22
Log2 Normalized Abundance
Log2 Size Class (uM)

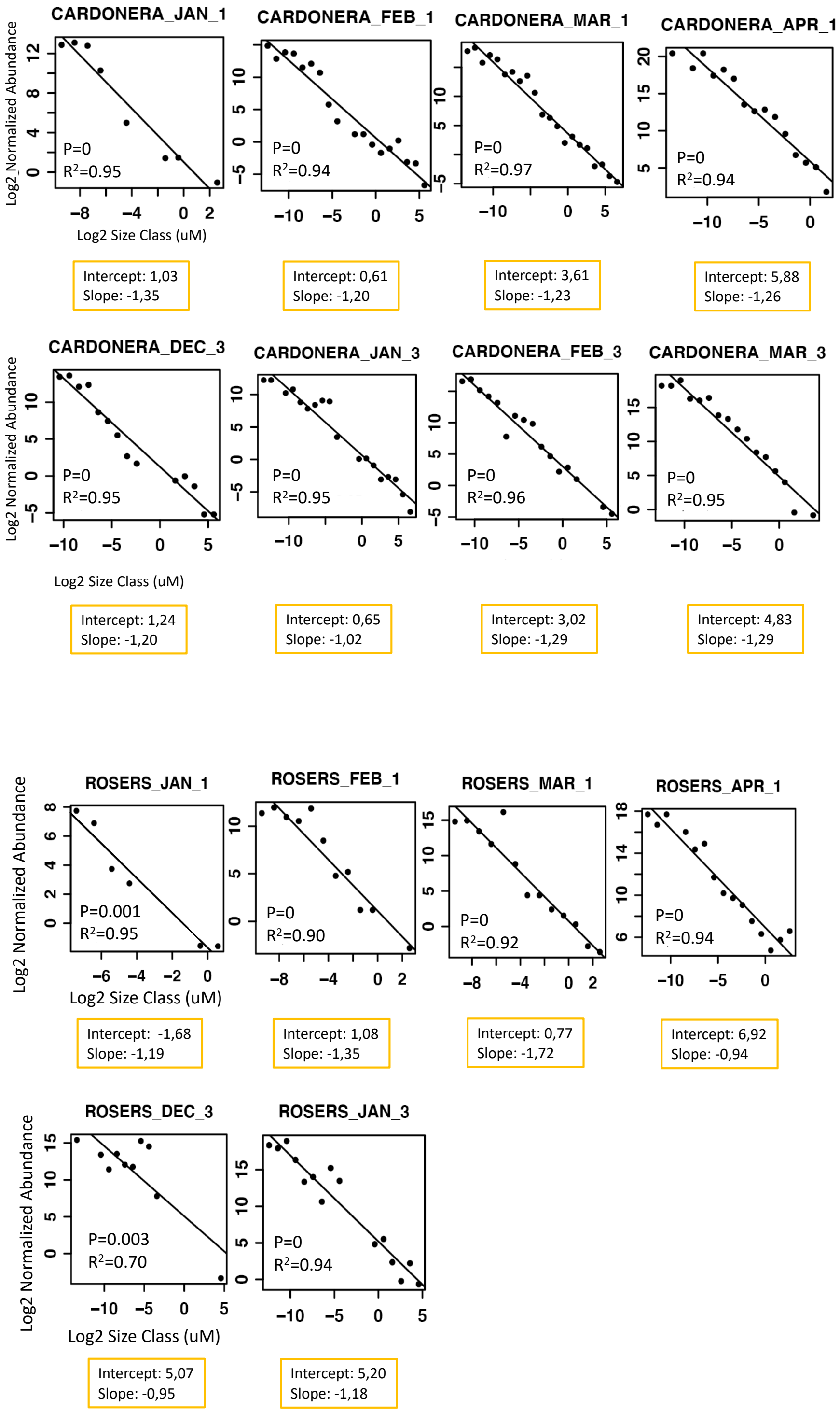


Figure S3. Ecological size spectra of zooplankton in each pond, for each month sampled during the three hydroperiods. On the bottom of each size spectrum, intercepts and slopes values are shown. The pond name is indicated on the top of each spectrum, together with the month. The number following the month indicates the hydroperiod (1,2,3).

Table S5. List of taxa found in Mediterranean brackish ponds (Chapters 1 and 2). In the first column the taxa are shown until the lower level of identification. 'Cl.' stays for 'Class', 'O.' stays for 'Order', and 'F.' stays for 'Family'. In the second column the ponds in which the previous taxa were found are shown. 'LLU' stays for 'Bassa de la llúdriga', 'CON' stays for 'Connectada', 'BPI' stays for 'Bassa del Pi', 'RUB' stays for 'La Rubina', 'BF' stays for 'Bassa del fartet', 'TU' stays for 'Túries', 'FR' stays for 'Fra Ramon', 'FAN' stays for 'Fangassos', 'CO' stays for 'Camping Out', 'CS' stays for 'Camping sud', 'CN' stays for 'Camping nord', 'AN' stays for 'Bassa de l'anguila', and 'BN' stays for 'Bassa nova'.

TAXA	POND
PHYLUM CHLOROPHYTA	
Cl. Prasinophyceae	
O. Pyramimonadales	
F. Pyramimonadaceae	
Pyramimonas sp.	LLU,CO,BPI,RUB
O. Volvocale	
F. Chlamydomonadaceae	
Chlamydomonas sp.	RUB
PHYLUM OCHROPHYTA	
Cl. Bacillariophyceae	
O.Cymbellales	
F. Cymbellaceae	
Cymbella sp.	BN,BF
O.Bacillariales	
F. Bacillariceae	
Nitzschia closterium	TU,LLU,BN,RUB,FR,CON
Pseudo-nitzschia sp.	BF
O.Naviculales	
F. Naviculaceae	
Navicula spp.	AN,TU,LLU,CO,BPI,BN,RUB,FR,FAN,CON
F. Pleurosigmataceae	
Gyrosigma spp.	TU,CO,CS,BPI,RUB,CON
F. Diploneidaceae	
Diploneis sp.	TU
O.Cocconeidales	
F. Cocconeidaceae	
Cocconeis placentula	AN,TU,LLU,BN,RUB,FAN,CN,BF,CON
O.Surirellales	
F. Entomoneidaceae	
Entomoneis sp.	BP,RUB,CON
O.Chaetocerotanae incertae sedis	
F. Chaetocerotaceae	
Chaetoceros sp.	BF

TAXA	POND
PHYLUM CRYPTOPHYTA	
Cl. Cryptophyceae	
O. Pyrenomonadales	
F. Pyrenomonadaceae	
Rhodomonas sp.	TU
F. Chroomonadaceae	
Hemiselmis sp.	CO,CS,BN
Chroomonas sp.	CN,CON
PHYLUM EUGLENOZOA	
Cl. Euglenoidea	
O. Euglenales	
F. Euglenaceae	
Euglena sp.	CO,BPI,FR
PHYLUM MYZOZOA	
Cl. Dinophyceae	
O. Gymnodiniales	
F. Gymnodiniaceae	
Gymnodinium spp.	AN,LLU,CO,CS,BPI,RUB,FR,FAN,CN,CON
Gyrodinium sp.	CS
Amphidinium sp.	TU
O. Oxyrrhinales	
F. Oxyrrhinaceae	
Oxyrrhis marina	AN,TU,LLU,BPI,BN,FR,FAN
O. Prorocentrales	
F. Prorocentraceae	
Prorocentrum micans	AN,TU,LLU,CS,CN,CON
O. Peridiniales	
F. Heterocapsaceae	
Heterocapsa sp.	BF
O. Thoracosphaerales	
F. Thoracosphaeraceae	
Scrippsiella subsalsa	FAN
O. Gonyaulacales	
F. Gonyaulacaceae	
Alexandrium sp.	CO,FR,BF
Alexandrium pseudogonyaulax	CS
PHYLUM CYANOBACTERIA	
Cl. Cyanophyceae	FR

TAXA	POND
PHYLUM ROTIFERA	
Cl. Rotatoria	
O. Ploimida	
F. Asplanchnidae	
Asplanchna sp.	CON,CS,CO
F. Lecanidae	
Lecane spp.	TU, RUB
Monostyla lamellata	BF
Monostyla bulla	FR,CN,CS
F. Brachionidae	
Keratella hiemalis	BN,CON,CN,AN
Keratella testudo	CS,CO
Lepadella sp.	BF,RUB
Brachionus plicatilis	BPI,FR,BF,TU,CS,CO,RUB
F. Trichocercida	
Trichocerca pusilla	CS
PHYLUM ARTROPODA	
Subphylum Crustacea	
Cl. Branchiopoda	
O. Cladocera	
F. Daphniidae	
Daphnia magna	TU
Cl Maxillopoda	
Sb. Cl. Copepoda	
O.Calanoida	
F. Diaptomidae	
Diaptomus sp.	FAN
F. Temoridae	
Eurytemora velox	FR
O.Harpacticopida	
F. Harpacticidae	
Harpacticus littoralis	TU, CS
F. Tisbidae	
Tisbe longicornis	TU, CS, FAN
F. Canuellidae	
Canuella perplexa	CON, AN, LLU
F. Canthocamp	
Cletocamptus sp.	FR

TAXA	POND
PHYLUM CHORDATA	
Cl. Actinopterygii	
O. Anguilliformes	
F. Anguillidae	
Anguilla anguilla	LLU
O. Cyprinodontiformes	
F. Cyprinodontidae	
Aphanius iberus	BPI,FR,BF,TU,CN,CS,CO,AN,LLU,FAN
F. Poecillidae	
Gambusia holbrooki	BN,FR,TU,CO,RUB
O. Atheriniformes	
F. Atherinidae	
Atherina boyeri	CN,CS,AN,LLU
O. Mugiliformes	
F. Mugilidae	
Mugil cephalus	CS,CON,AN
O. Perciformes	
F. Centrarchidae	
Lepomis gibbosus	BN,AN,LLU
F. Gobiidae	
Pomatoschistus sp.	TU,CON,CN,CS,AN,LLU
O. Pleuronectiformes	
F. Soleidae	
Solea solea	LLU

Table S6. List of taxa found in Mediterranean temporary freshwater ponds (Chapters 3). In the first column the taxa are shown until the lower level of identification. 'Cl.' stays for 'Class', 'O.' stays for 'Order', and 'F.' stays for 'Family'. The other three columns represent where those taxa were found: Rajoleria pond, Cardonera pond, and Prats del Roser pond respectively, represented also by circles of different colours (red for Rajoleria, blue for Cardonera, and yellow for Prats del Roser), for a more visual interpretation of the data. The numbers represent the month of the year in which taxa were found. '1' stays for January, as the first month of the year, '2' stays for February, '3' for March and so on until '12' which stays for December. No distinction among the three hydroperiods studied is shown. The letter in parenthesis shown along with the months (when presents), represent the developmental stage of the taxa found. 'L' stays for 'Larvae', 'A' stays for 'Adult', 'N' stays for 'Nimph', and 'P' for 'Pupa'. In addition, the taxa in bold show which are the macroinvertebrates considered in this thesis as potential predators for zooplankton.

TAXA	POND RAJOLERIA	POND CARDONERA	POND PRATS DEL ROSER
PHYLUM CHLOROPHYTA			
Cl. Trebouxiophyceae			
O. Chlorellales			
F. Oocystaceae			
Oocystis sp.	🔴 2,4,6,12	🔵 3	
Cl. Chlorophyceae			
O. Oedogoniales			
F. Oedogoniaceae			
Oedogonium		🔵 3	
O. Sphaeropleales			
F. Selenastraceae			
Ankistrodesmus sp.	🔴 2,3,12	🔵 2,3	
Monoraphidium sp.	🔴 4	🔵 2,3,4	
Monoraphidium contortum		🔵 2	
F. Scenedesmaceae			
Scenedesmum sp.	🔴 5		🟡 3
Scenedesmum acutus		🔵 4	🟡 4
Scenedesmum arcuatus			🟡 4
O. Volvocale			
F. Chlamydomonadaceae			
Chlamydomonas spp.	🔴 1,2	🔵 1,2,3,4,12	🟡 1,2
F. Volvocaceae			
Eudorina sp.	🔴 1,2,11		🟡 2
Volvox spp.	🔴 1,2,3,11,12		
PHYLUM OCHROPHYTA			
Cl. Chrysophyceae			
O. Chromulinales			
F. Dinobryaceae			
Dinobryon sp.	🔴 1		
Cl. Xanthophyceae			
O. Mischococcales			
F. Centritractaceae			
Centritractus belenophorus	🔴 1	🔵 1	
O. Tribonematales			
F. Tribonemataceae			
Tribonema spp.	🔴 1,2,3,4,11,12	🔵 2,3	
Cl. Bacillariophyceae			
O.Cymbellales			
F. Cymbellaceae			
Cymbella sp.	🔴 1,4,5		
F. Gomphonemataceae			
Gomphonema sp.		🔵 3	🟡 4
O.Bacillariales			
F. Bacillariceae			
Nitzschia sp.	🔴 1,2,3,5,12	🔵 3	🟡 4
Fragilariopsis sp.	🔴 1,12		
Cylindrotheca closterium	🔴 1,2,3,12		
O.Naviculales			
F. Naviculaceae			
Navicula sp.	🔴 1,11	🔵 2,3	🟡 1
F. Stauroneidaceae			
Stauroneis sp.	🔴 5		
F. Pinnulariaceae			
Pinnularia sp.	🔴 2,5,11	🔵 2	

TAXA	POND RAJOLERIA	POND CARDONERA	POND PRATS DEL ROSER
PHYLUM CRYPTOPHYTA			
Cl. Cryptophyceae			
O. Cryptomonadales			
F. Cryptomonadaceae			
Cryptomonas spp.	● 1,2,3,4,5,6,11,12	● 1,2,3,4,12	● 1,4,12
PHYLUM EUGLENOZOA			
Cl. Euglenoidea			
O. Euglenales			
F. Euglenaceae			
Trachelomonas spp.	● 1,2,3,4,5	● 1,3,4,12	● 1,2,3,4,5,12
Euglena sp.	● 1,3,4	● 1,2,3,4	● 1,2,3,4,12
Euglena oxyuris	● 5		
F. Phacaceae			
Phacus sp.	● 1,4,5	● 4	
PHYLUM CYANOBACTERIA			
Cl. Cyanophyceae			
O. Nostocales			
F. Nostocaceae			
Anabaena spp.	● 1,2,3,4	● 1,3	● 1,4
PHYLUM MYZOZOA			
Cl. Dinophyceae			
O. Gymnodiniales			
F. Gymnodiniaceae			
Gymnodinium spp.	● 2,4,5	● 3	● 1,2,3
O. Peridiniales			
F. Peridiniaceae			
Peridinium sp.	● 2,3		● 1,3
F. Peridiniopsisdaceae			
Peridiniopsis sp.	● 2,5		● 2,3,4,12
PHYLUM CHAROPHYTA			
Cl. Zygnematophyceae			
O. Zygnematales			
F. Desmidiaceae			
Staurodesmum glaber	● 12		
Staurodesmum extensus	● 4		
Cosmarium spp.	● 1,2,3,4,5,12		● 2
Xanthidium bifidum			● 2
F. Closteriaceae			
Closterium spp.	● 3,4		● 3,4
F. Zygnemataceae			
Spyrogira sp.	● 2,3		
PHYLUM ROTIFERA			
Cl. Rotatoria			
O. Ploimida			
F. Asplanchnidae			
Asplanchna sp.	● 1,2,3,5,12	● 1,3	
F. Lecanidae			
Lecane sp.	● 3,12		● 2,3
Lecane luna	● 1,5		
Monostyla sp.	● 1,2,3,4,12	● 3	● 1,2,4
Monostyla bulla	● 4		● 4
F. Brachionidae			
Euchlanis sp.		● 1	● 2,4
Euchlanis dilatata	● 1,3,11,12	● 1,2,3	
Keratella cochlearis	● 11		
Keratella hiemalis	● 1,2,3,4,5,6,11,12	● 1,4,12	● 1,2,3,12
Keratella valga	● 5	● 3	
Lepadella sp.		● 1,12	● 2,12
Lepadella patella	● 1,2,3,4,6		
Brachionus sp.			● 3
Brachionus quadridentatus	● 1	● 2,3	
Anuraeopsis sp.			● 2,3
Trichotria tetractris	● 1,2,11,12	● 1,2,3,12	
Platyias quadricornis	● 3		
F. Gastropodidae			
Gastropus sp.	● 11		
Ascomorpha sp.	● 2,4,11,12	● 1	
Ascomorpha ecaudis	● 3		
F. Synchaetidae			
Bipalpus hudsoni	● 12		
Synchaeta sp.	● 12		
Polyarthra sp.	● 1,4,5,12	● 3,4	● 1,3,4,12
F. Trichocercidae			
Trichocerca sp.	● 1,2,3,11	● 3	● 1,12
Trichocerca cylindrica	● 12		
Trichocerca rattus	● 1		
Trichocerca similis	● 1,4,12		
O. Gnesiotrocha			
F. Conochilidae			
Conchilus sp.	● 1,2,3,5,6,12	● 2,3,12	
F. Collothecidae			
Collotheca sp.	● 2,3		
O. Flosculariacea			
F. Testudinellidae			
Pompholyx sulcata		● 2	

TAXA	POND RAJOLERIA	POND CARDONERA	POND PRATS DEL ROSER
PHYLUM ARTROPODA			
Subphylum Crustacea			
Cl. Branchiopoda			
O. Cladocera			
F. Daphniidae			
Simocephalus vetulus	● 1,2,4,12	● 3	● 3
Daphnia curvirostris	● 1		
F. Moinidae			
Moina braquiata	● 4,5,6		
Moina micrura		● 4	● 4
F. Eurycercidae			
Chydorus sphaericus	● 1,2,3,4,12	● 2,3,4	
Alonella sp.	● 1,2		
Alonella excisa	● 4,6		
Alonella nana	● 6		
Pleuroxus denticulatus	● 1,2,4		
Treptocephala ambigua	● 1,3,4,11,12	● 3	
Cl Maxillopoda			
Sb. Cl. Copepoda			
O.Calanoida			
F. Diaptomidae			
Mixodiaptomus kupelwiesseri	● 2,5,6,12	● 2	
Mixodiaptomus incrassatus	● 1,11,12		● 3
Diaptomus cyaneus	● 1,2,5,12	● 1,2,3,12	● 1,12
O.Cyclopoida			
F. Cyclopidae			
Acanthocyclops viridis	● 1,2	● 1,3	● 1
Acanthocyclops gigas	● 1,3		● 4
Megacyclops viridis	● 4,5		
Diacyclops nanus		● 4	
Diacyclops bicuspidatus	● 1,12		● 1,3
O.Harpacticopida			
F. Canthocamptidae			
Canthocamptus staphilinus	● 1,2,3,4,6,11,12	● 1,3	● 2
Canthocamptus microstaphilinus	● 11		
O. Amphipoda			
F. Niphargidae (L)	● 2	● 1,2,12	
O. Anostraca			
F. Chirocephalidae			
***Chirocephalus* sp.**	● 1 (A),2(A),12(L)	● 1 (L),2 (L+A),12 (L)	
Subphylum Tracheata			
Cl. Insecta			
O. Coleoptera			
F. Hygrobiidae (L)	● 1,5,6,12	● 4	
***Hygrobia* sp.**	● 2 (L+A),12 (L)	● 1 (A),3 (A)	● 1 (A)
F. Hydrophilidae	● 1 (L+A),2(L+A),3(A),5(L),6(L)	● 1(L),3(L),4(P)	● 1(L),4(L)
Berosus sp.	● 5 (A)		
Helophorus sp.	● 4 (A)		
F. Dryopidae (L)	● 4		
F. Dytiscidae (L)	● 1,2,3,5,6,12	● 1,2,3,12	● 1,3,4
Rhantus sp. (A)	● 5		
F. Elmidae	● 3 (A)	● 1 (L),12 (L)	
F. Gyrinidae			
***Gyrinus* sp. (A)**	● 4,6	● 4	● 4
F. Haliplidae	● 2 (L+A),3 (L)	● 3 (A)	
F. Hydraneidae (L)	● 12		
O. Diptera			
F. Chironomidae	● 1(L+P),2(L),3(L+P),5(L)	● 2(L),3(L+P),4(L)	● 3(L+P),4(L+N)
Sub F.Tanypodinae (L)	● 1,2	● 3,4	
F. Culicidae		● 3(L)	● 1(L+P),3(L),4(L)
F. Chaoboridae	● 5 (L)		
F. Simuliidae	● 2 (L), 3(L)		
O. Ephemeroptera			
F. Baetidae (L)	● 6		● 4
F. Potamanthidae			
Pothamantus sp.	● 3		
O. Hemiptera	● 1 (A)	● 1 (A)	
F. Pleidae	● 3 (L)		
Sub.O. Heteroptera			
F. Corixidae	● 6 (L+A)	● 4 (A)	● 4 (A+N)
***Micronecta* sp.**	● 1 (A),3 (A)	● 12 (A)	
F. Notonectidae			
***Notonecta* sp.**	● 1(A),2(A),4(A),5(A),6(A),12(L)	● 3(A),4(A)	● 1(L),4(A)
F. Gerridae			
Gerris sp.	● 5 (A)		● 4 (A+N)
O. Odonata			
F. Libellulidae (L)	● 3,4,5,6,		
F. Lestidae (L)	● 5,6		
O. Trichoptera (L)	● 1		
F. Hydropsychidae (L)	● 12		

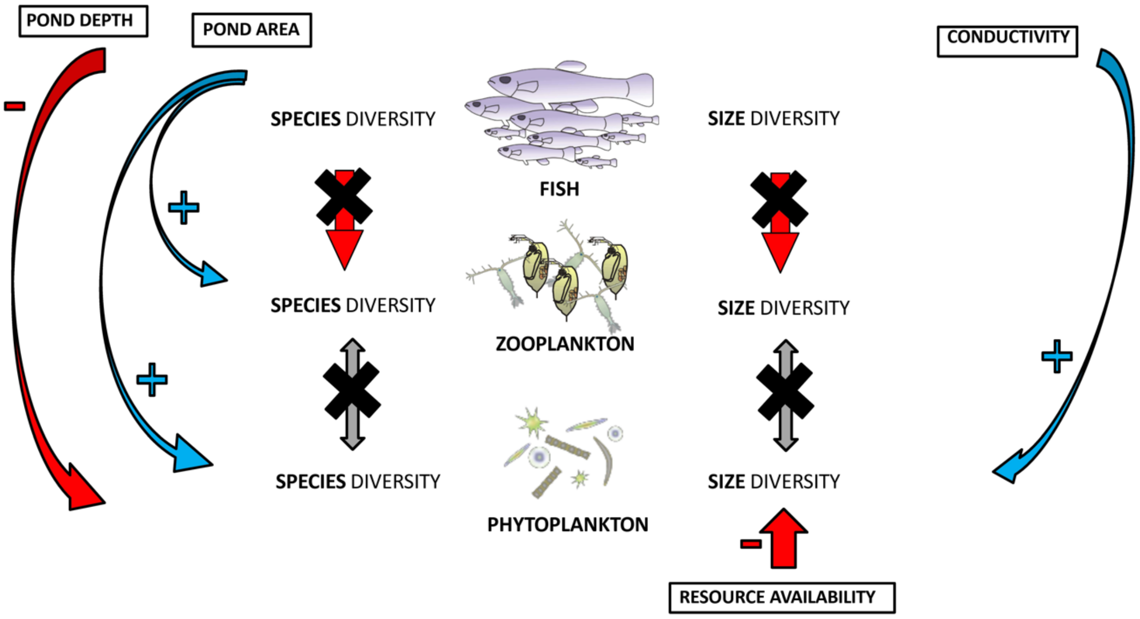


Figure S4. Graphical abstract of Chapter 1. In the middle the three trophic levels considered are shown (size diversity on the right side and species diversity on the left side). The arrows specify the direction of the effect, being the head of the arrow the response variable and the tail of the arrow, the predictor variable. The black cross states a lack of interaction between the variables, whereas the '+' and '-'symbols mean if the correlation between them is positive or negative.

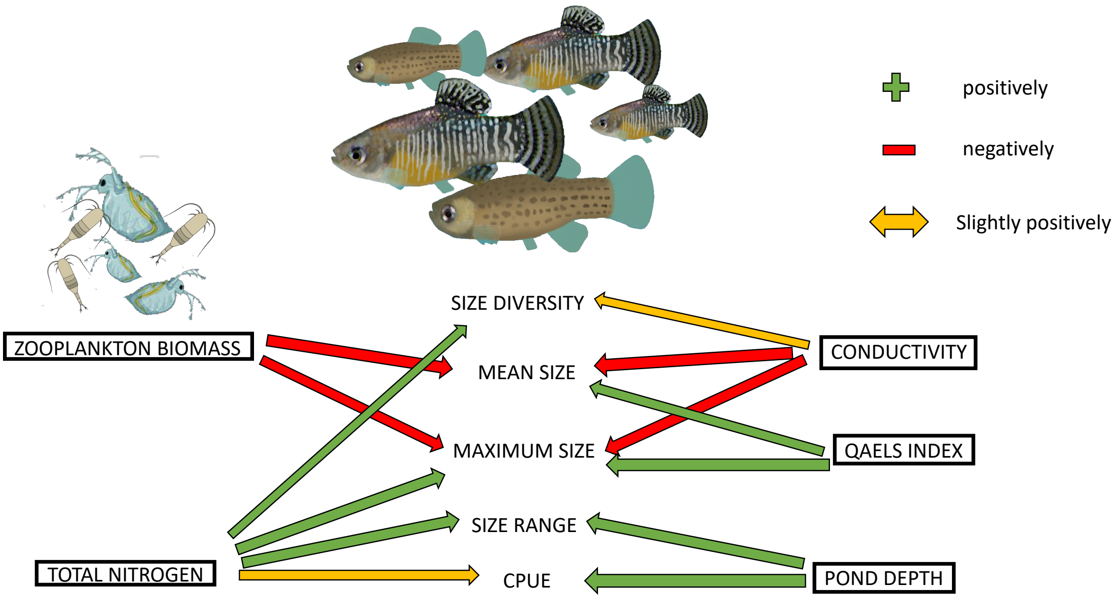


Figure S5. Graphical abstract of Chapter 2. In the middle, *A. iberus*' size metrics and abundance (CPUE). The arrows specify the direction of the effect, being the head of the arrow the response variable and the tail of the arrow, the predictor variable. The colour of the arrow shows if the correlation between them is positive (green), negative (red), or slightly positive (yellow).

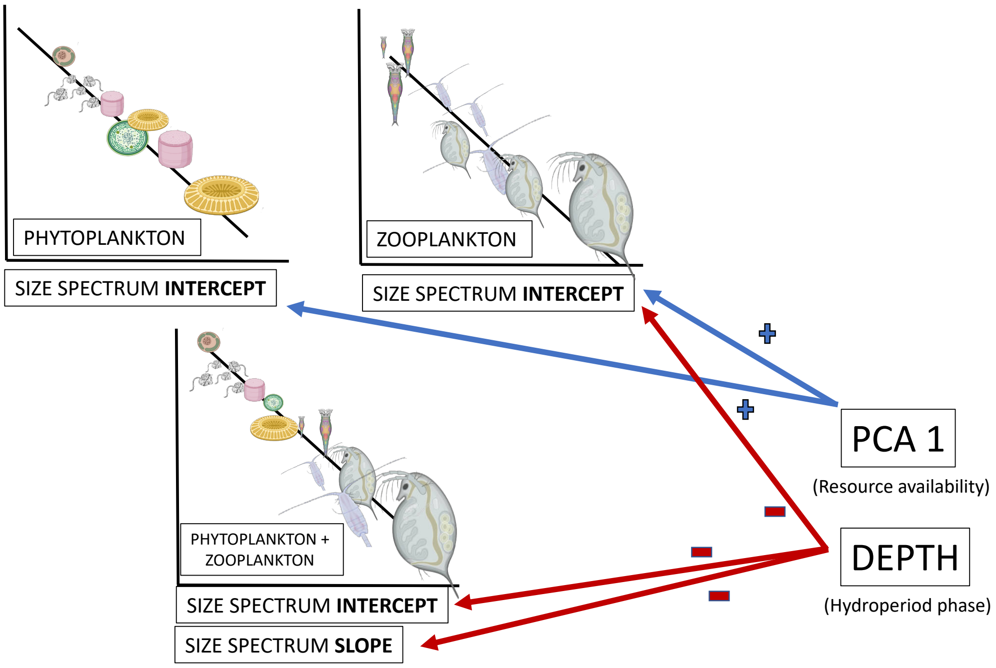


Figure S6. Graphical abstract of Chapter 3. Size spectrum intercept and slope of the communities considered in the study (phytoplankton, zooplankton, phytoplankton + zooplankton) are shown, together with the abiotic factors resulted to be important in shaping planktonic size structure. The arrows specify the direction of the effect, being the head of the arrow the response variable and the tail of the arrow, the predictor variable. The '+' and '-' symbols mean if the correlation between them is positive or negative.

12. OTHER PUBLICATIONS BY THE AUTHOR

Ersoy, Z., Jeppesen, E., Sgarzi, S., Arranz, I., Cañedo-Argüelles, M., Quintana, X. D., ... & Brucet, S. (2017). Size-based interactions and trophic transfer efficiency are modified by fish predation and cyanobacteria blooms in Lake Mývatn, Iceland. *Freshwater Biology*, *62*(11), 1942-1952.
https://doi.org/10.1111/fwb.13039

Cañedo-Argüelles, M., Sgarzi, S., Arranz, I., Quintana, X. D., Ersoy, Z., Landkildehus, F., ... & Brucet, S. (2017). Role of predation in biological communities in naturally eutrophic sub-Arctic Lake Mývatn, Iceland. *Hydrobiologia*, *790*(1), 213-223.
https://doi.org/10.1007/s10750-016-3031-0

Bartrons, M., Arranz, I., Cañedo-Argüelles, M., Sgarzi, S., Lauridsen, T. L., Landkildehus, F., ... & Jeppesen, E. (2018). Fish shift the feeding behaviour and trophic niche diversification of their prey in subarctic Lake Mývatn, Iceland. *Hydrobiologia*, *816*(1), 243-254.
https://doi.org/10.1007/s10750-018-3588-x

13. ORAL PARTICIPATIONS IN CONFERENCES

Sgarzi, S., Cañedo, M., Arranz, I., Quintana, X.D., Ersoy, Z., Jeppesen, E., Brucet, S. Role of predation in biological communities in naturally eutrophic subarctic Lake Mývatn (Iceland), AIL 2016, 4-8 July 2016, Tortosa, Spain.

Sgarzi, S., Badosa, A., Benejam, L., Leiva A., Arranz, I., Bartrons, M., Ersoy, Z., Brucet, S. Size-based interactions in pelagic trophic webs of Mediterranean freshwater and brackish ponds, SEFS 2017, 2-7 July 2017, Olomouc, Czech Republic.

Ersoy, Z., Jeppesen, E., **Sgarzi, S**., Quintana, X.D., Arranz, I., Cañedo, M., Landkildehus, F., Lauridsen T.L., Einarsson, Á., Brucet, S. Size-based interactions within the food web of Lake Mývatn, Iceland: a mesocosm experiment, SIL Congress, 31 July-5 August 2016, Torino, Italy.

Brucet, S., Ersoy, Z., Jeppesen, E., **Sgarzi, S.,** Quintana, X.D., Arranz, I., Cañedo, M., Landkildehus, F., Lauridsen T.L., Einarsson, Á. Size-based interactions across trophic levels of the planktonic food web: a lake mesocosm experiment, AIL 2016, 4-8 July 2016, Tortosa, Spain.

Sgarzi, S., Badosa, A., Leiva-Presa, A., Bartrons, M., Ersoy, Z., López, E., Brucet, S. Size spectra of phytoplankton and zooplankton in Mediterranean temporary ponds along the hydroperiod gradient, SIL 2021, 22-27 August 2021, Gwangju, Republic of Korea (online sessions).