

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

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ABSTRACT

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The biogeographical principle that larger areas contain more species than smaller areas has more often been assumed than tested. In this sense, contradictory results have been published in studies on the relationship between water body size and species richness in temporary waters. Pond size can have an effect on the structure and composition of the macrofauna community, but this effect can be modified by other environmental factors such as water trophic state, habitat structure and spatial connectivity within the ecosystem. We sampled the aquatic macrofauna (from midges to amphibians) from a network of twelve Mediterranean temporary ponds in southwestern Portugal with a strong size gradient (245-78 652 m²), also taking into account three environmental factors that can modulate the relationship between pond size and community structure: connectivity, water trophic state and habitat structure. Our aim was to test the importance of pond size in macrofaunal structure and composition. Pond size was not related to any of the three environmental factors included in this study. Our results noted an unclear relationship between pond size and macrofauna, since we found a significant effect on community composition but did not find an effect on community structure parameters such as richness, taxonomic diversity or body size diversity. The high connectivity among ponds seems to be a plausible explanation for the observed pattern.

Key words: Ecosystem size, metacommunity, Mediterranean temporary ponds, macroinvertebrates, species richness, connectivity.

RESUMEN

Efecto del tamaño de la laguna en la estructura de la comunidad de macrofauna en una red de lagunas altamente conectadas

El principio biogeográfico que establece que áreas de mayor tamaño tienen más especies que áreas menores ha sido en ocasiones más asumido que comprobado. En este sentido, se han publicado resultados contradictorios en estudios sobre la relación entre el tamaño de la masa de agua y la riqueza de especies en aguas temporales. El tamaño de la laguna puede tener un efecto en la estructura y composición de la comunidad de macrofauna, pero este efecto puede verse modificado por otros factores ambientales tales como el estado trófico del agua, la estructura del hábitat y la conectividad espacial dentro del ecosistema. Muestreamos la macrofauna acuática (desde quironómidos hasta anfibios) de una red de doce lagunas temporales mediterráneas en el suroeste de Portugal con un fuerte gradiente de tamaños (245-78 652 m²) teniendo también en cuenta tres factores ambientales que pueden modular la relación entre el tamaño de la laguna y la estructura de la comunidad: conectividad, estado trófico del agua y estructura del hábitat. Nuestro objetivo era comprobar la importancia del tamaño de la laguna sobre la estructura y la composición de la macrofauna. El tamaño de la laguna no presentó relación con ninguno de los tres factores ambientales incluidos en este estudio. Nuestros resultados mostraron una relación poco clara entre el tamaño de la laguna y la macrofauna ya que encontramos un efecto significativo en la composición de la comunidad, pero no encontramos un efecto en los parámetros de estructura de la comunidad tales como la riqueza, la diversidad taxonómica o la diversidad del tamaño corporal. La alta conectividad entre las lagunas parece ser una explicación verosímil para el patrón observado.

Palabras clave: Tamaño del ecosistema, metacomunidad, lagunas temporales mediterráneas, macroinvertebrados, riqueza de especies, conectividad.

INTRODUCTION

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

(Spencer *et al.*, 1999; McCann *et al.*, 2005; Arim *et al.*, 2010).

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

lated (Olden *et al.*, 2001). For instance, in the case of amphibians, both landscape and pond connectivity are very important for the long term persistence of their populations because they may buffer the stochastic events that frequently occur in Mediterranean ponds (Ribeiro *et al.*, 2011). In the case of macroinvertebrates, the connectivity between ponds can increase the similarities between communities compared to non-connected or indirectly connected ponds (Van de Meutter *et al.*, 2007).

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

size effect on the structure and composition of the macrofauna. To test this, we first studied the relationships between each of the three environmental factors (water trophic state, habitat structure and connectivity) and pond size, and we then established the relationships between community structure parameters and composition and pond characteristics.

MATERIALS AND METHODS

Study site

The study was carried out in twelve temporary ponds situated near the village of Vila Nova de Milfontes (37°45'N, 8°48'W) within the Southwest Alentejo and Vicentine Coast Natural Park in Portugal (Fig. 1). These twelve ponds were intentionally chosen to include the broadest possible range of sizes available in the study area (245-78 652 m²). They are located on a coastal

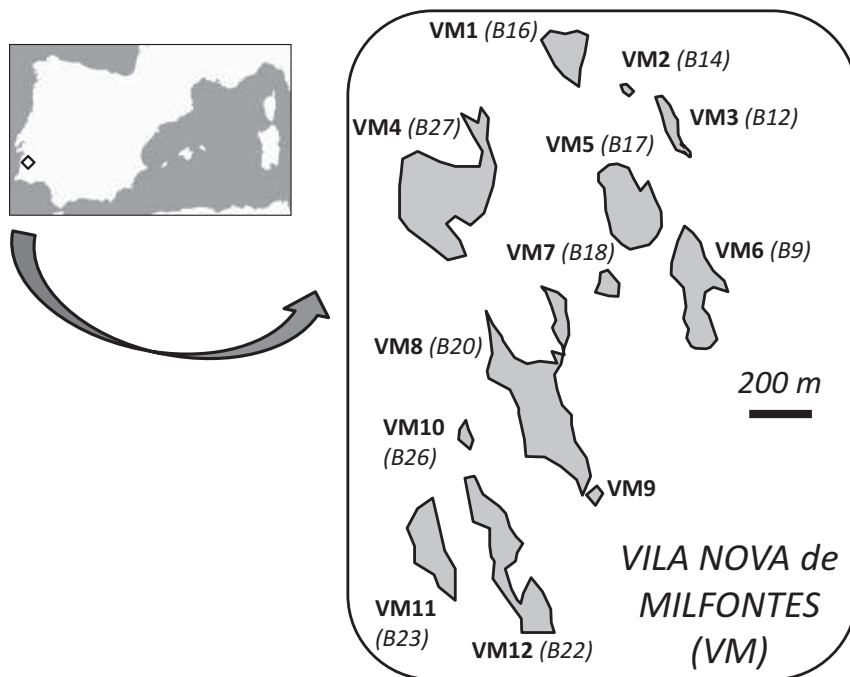


Figure 1. Study site locations showing pond positions within the network. The original codification of the ponds used by Caramujo & Boavida (2010) and Chaves (1999) is shown in brackets. *Localización de la zona de estudio mostrando la posición de las lagunas que conforman la red. La codificación original de las lagunas usada por Caramujo & Boavida (2010) y Chaves (1999) se muestra entre paréntesis.*

sandy plateau protected by consolidated dunes on the west and by a wooded area to the east (Caramujo & Boavida, 2010). This is a set of ponds of different sizes, shapes and depths that in turn show variations in these features individually throughout the year (Chaves, 1999). Although there is intra-annual and inter-annual variation in the duration of the wet phase (i.e., hydroperiod length), the ponds are usually filled in November-December and start to dry in March (Caramujo & Boavida, 2010; Martins *et al.*, 2010). The climate is Mediterranean with an oceanic influence. The soil is highly permeable, and the ponds fill mainly with rain water (Caramujo & Boavida, 2010), although they are also possibly fed by groundwater (Chaves, 1999). Although we did not study the hydroregime of the ponds, according to Chaves (1999), who sampled the aquatic insects of these ponds during the entire hydroperiod, we can infer that the ponds dry out between May and June. The three-phases model in succession patterns has long been established, with the first and the last phases (flooding and drying phases, respectively) being characterised by drastic changes in the community structure (Boix *et al.* 2016). Because we wanted to sample the community avoiding periods when such changes occur, we thought April was a suitable time to conduct this study.

Water characteristics

Water temperature (T), conductivity (Cond), pH and dissolved oxygen (O₂) were measured *in situ* using a Hach HQ30d portable multi-parameter metre. Filtered water samples (250 mL) and unfiltered water samples (250 mL) were collected from each pond and were frozen immediately. The dissolved inorganic nutrients (ammonia, nitrite, nitrate, phosphate) were measured from the filtered water samples with the ion chromatography system Dionex ICS-5000. DIN (dissolved inorganic nitrogen) was then calculated as the sum of the concentrations of ammonia, nitrite and nitrate. The total nutrients (total nitrogen (TN-N) and phosphorus (TP-P)) were analysed from the unfiltered water samples, following Grasshoff *et al.* (1983). The dissolved organic

carbon (DOC), total organic carbon (TOC), dissolved inorganic carbon (DIC) and total inorganic carbon (TIC) were analysed using the TOC analyser Shimadzu TOC-V CSH and following the UNE-EN 1484: 1998 guidelines. The planktonic chlorophyll *a* (Chl_a) content was extracted using 90% acetone after filtering the water samples (Whatman GF/F filters). The chlorophyll *a* analyses were carried out with high pressure liquid chromatography (HPLC; Waters pump (1500 Series) with an autosampler injector (Waters 717 Plus) and a diode-array detector (Waters PDA 2996) using an adaptation of the method of Zapata *et al.* (2000), with a C8 reverse phase column and a pyridine mobile phase). A nutrient limitation indicator was assessed using the ratio between the DIN and the TP (molar DIN/molar TP). Values of the ratio below 2 indicate N limitation, and values above 5 indicate P limitation (Ptacnik *et al.*, 2010). To determine the fulvic acids content, a modification of the method described by Hautala *et al.* (2000) was used. The samples were acidified to pH < 2.5 with 1N HCl. Twenty-four hours after the acidification, the samples were filtered through a Whatman GF/C filter to eliminate the precipitates of humic acids. The fulvic acids concentration was obtained through spectrophotometry at 350 nm using a UV-1600PC spectrometer (Model VVVR) and applying the regression described in Gan *et al.* (2007).

Sampling procedure and processing

The survey was undertaken between the 22nd and 23rd of April, 2013, taking one sample from each pond. The macrofauna samples were taken using a dip net with a diameter of 22 cm and a mesh size of 250 µm. The sampling procedure was based on 20 dip-net sweeps in rapid sequence that spanned all of the different mesohabitats. The implemented sampling procedure attempted to solve two problems that we have to face when sampling ponds of different sizes. On one hand, if the same sampling effort is applied in all the ponds, the samples taken from the smallest ponds will reflect the spatial heterogeneity better than those from the largest ponds. On the other hand, if a greater sampling effort is applied,

the comparison of the samples is not reliable because, as some authors have indicated (Gaston & Spicer, 2004; Azovsky, 2010), the measures of the community structure change with sampling effort. Thus, to remove this “sampling effort effect”, some authors apply a different sampling effort depending on the pond size (e.g., Oertli *et al.*, 2002; Søndergaard *et al.*, 2005). In the current study, we followed the following procedure: we applied the same sampling effort (20 dip-net sweeps) in all of the ponds, although the sample from each pond was obtained from a different number of dip-net sweeps that depended on the pond size. Thus, in small ponds (200-2500 m²), the sample was obtained by means of 20 dip-net sweeps. In medium ponds (> 2500-20 000 m²), 40 dip-net sweeps were conducted; then, the capture was homogenised, and only half of this (equivalent to 20 dip-net sweeps) constituted the sample. Finally, in the big ponds (> 20 000 m²), 60 dip-net sweeps were performed; the capture was then homogenised, and one third (equivalent to 20 dip-net sweeps) of the total capture constituted the sample, with the rest being released into the water. The samples were preserved *in situ* in 96% ethanol.

Subsequently, in the laboratory, the preservative of the samples was removed, and the individuals were sorted, counted and identified to the species level whenever possible, except in the case of chironomids, which were identified to the subfamily. At least 26 individuals for each taxon and sample were randomly chosen for measurements to estimate individual biomass.

Pond size, connectivity, habitat structure and water trophic state determination

The maximum surfaces of the different ponds were estimated using the Google Maps Area Calculator Tool (Daftlogic, 2015) and were posteriorly checked in the field. The spatial connectivity between ponds was measured using the equation in Henriques-Silva *et al.* (2013):

$$\text{Average Connectivity} = \frac{1}{n} \sum_{\substack{i=1 \\ i \neq j}}^n p_{jk} \exp(-d_{ij})$$

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

We used the macrophyte biomass (Macrophytes_DW) per pond as a proxy for the habitat structure of each pond (e.g., Thomaz & Cunha, 2010). The macrophyte biomass (g DW/m²) was estimated as the mean dry weight of three replicates of 50.26 cm² that were taken randomly from each pond. The dry weight was obtained after oven-drying the material at 60 °C over 48 hours. Finally, to determine the trophic state of the system, TRIX (the trophic index) was calculated for each pond. This index is based on the planktonic chlorophyll *a*, oxygen saturation, total nitrogen and phosphorus. Numerically, the index is scaled from 0 to 10, covering a wide range of trophic conditions from oligotrophy (0) to eutrophy (10) (Vollenweider *et al.*, 1998). It was calculated using the following equation:

$$\text{Trophic Index} = \frac{(\log [Ch * aD\%O * N * P] - [-1.5])}{1.2}$$

where *Ch* is the chlorophyll *a* (mg/m³), *aD%O* is the oxygen as its absolute deviation (in percentage) from saturation (abs |100 - %O|), *N* is the total nitrogen (mg/m³) and *P* is the total phosphorus (mg/m³).

Community structure parameters

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

of the species in each sample. The IFO was calculated according to Puchalski (1987):

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

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

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

et al. (2008). This measure takes the form of an integral involving the probability density function of the body size of the individuals described by the following equation:

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

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

Statistical analyses

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

To analyse if the community structure parameters responded to pond size, simple linear regression models were performed. Previously, we had tested the assumptions of normality and homoscedasticity using the R function 'mcheck'. Pond size was the explanatory variable, and the

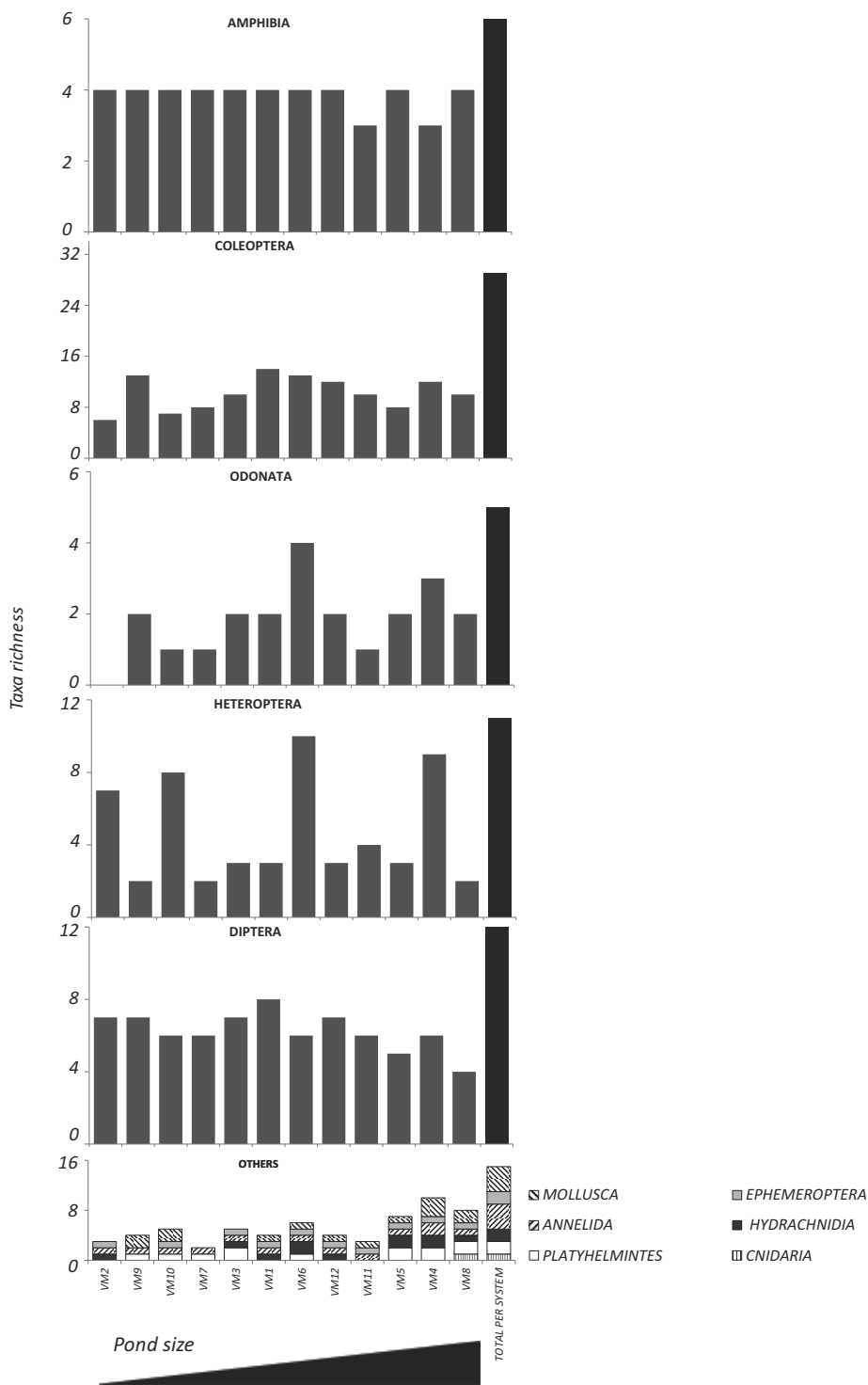


Figure 2. Taxa richness for each major group of organisms in each pond. Right bar indicates the total taxa richness per system. Pond size increases from left to right. *Riqueza de taxones para cada uno de los principales grupos de organismos en cada laguna. La barra de la derecha indica la riqueza total de taxones por sistema. El tamaño de las lagunas aumenta de izquierda a derecha.*

Table 1. Mean and range of variation of the physical and chemical characteristics of the ponds studied. *Media y rango de variación de las características físicas y químicas de las lagunas estudiadas.*

Water characteristics	Mean (Range)
Maximum depth (cm)	62.33 (31-106)
Temperature (°C)	21.53 (17.40-23.90)
Conductivity (µS/cm)	746.53 (390-1274.67)
pH	6.43 (5.36-7.28)
Dissolved oxygen (mg/L)	4.58 (2.82-6.61)
Ammonium (mg NH ₄ ⁺ -N/L)	0.02 (0.001-0.062)
Nitrate (mg NO ₃ ⁻ -N/L)	0.003 (0.002-0.009)
Nitrite (mg NO ₂ ⁻ -N/L)	0.004 (0.003-0.006)
Phosphate (mg PO ₄ ³⁻ -P/L)	0.02 (0.004-0.096)
Total nitrogen (mg NT-N/L)	2.77 (1.87-3.80)
Total phosphorus (mg PT-P/L)	0.13 (0.03-0.64)
Chlorophyll <i>a</i> (µg/L)	10.47 (0.27-40.87)
Trophic state index	4.96 (3.89-7.27)
DIC (mg C/L)	12.96 (2.06-73.64)
TIC (mg C/L)	14.30 (3.57-75.19)
DOC (mg C/L)	54.82 (40.14-79.44)
TOC (mg C/L)	57.67 (40.14-80.12)
molar DIN/molar TP	0.67 (0.10-2.27)
Macrophyte biomass (g DW/cm ²)	0.19 (0.12-0.31)
% fulvic acids	65.04 (50.89-86.49)

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

RESULTS

The macrofauna found in the entire pond network included 78 taxa, most of which were insects (59 taxa). The best represented orders of insects were Coleoptera (29 taxa), Diptera (12 taxa) and Heteroptera (11 taxa; for more details on the fauna, see Tornero *et al.*, 2014). When analysing the taxa richness of each pond for the major taxonomic groups, we detected that the amphibian and dipteran richness was almost the same among the ponds. In contrast, the richness of heteropterans, odonates and coleopterans was more variable. The greatest coleopteran richness was found in the ponds of intermediate size. Pond VM6, with an intermediate size, presented the greatest richness both in heteropterans and odonates (Fig. 2).

The first two axes of the PCA explained 52.3% of the variance. PC1 explained 25.3% of the variance, while PC2 explained 20.2% (Fig. 3). The variables with the highest contribution to PC1 were the TN-N, mean temperature (T), maximum depth (Depth) and conductivity (Cond). pH, DIC, TIC and DIN were the main variables contributing to PC2. Ponds VM11 and VM12 had the most distinct physical and chemical characteristics in relation to the others, as shown in the PCA plot (Fig. 3). Moreover, VM12 had the highest trophic conditions (TRIX index = 7.3; Table 1). In general, the system can be considered to be limited by N, with low values of pH, dissolved oxygen and DIN. However, it had high values of phosphates, TN, TOC, fulvic acids and macrophyte biomass.

In contrast, when looking at the relationships between the pond community composition similarities (NMDS) and the environmental factors analysed, pond size was a unique variable, showing a significant relationship ($p = 0.006$; Fig. 4A). The abundance of some taxa, such as *Gyraulus laevis* (Planorbidae), *Piona* sp. (Pionidae) and *Aeshna mixta* (Aeshnidae), increased in larger ponds, whereas the abundance of other taxa, such as *Agabus* sp. (Dytiscidae), *Chaoborus flavicans* (Chaoboridae) and *Culex theileri* (Culicidae), had their maximum density in smaller ones (Fig. 4B).

The rest of the variables (connectivity, TRIX, macrophyte biomass) did not show any relationships with the community composition data. In looking at the community structure parameters (regression results), no significant relationships

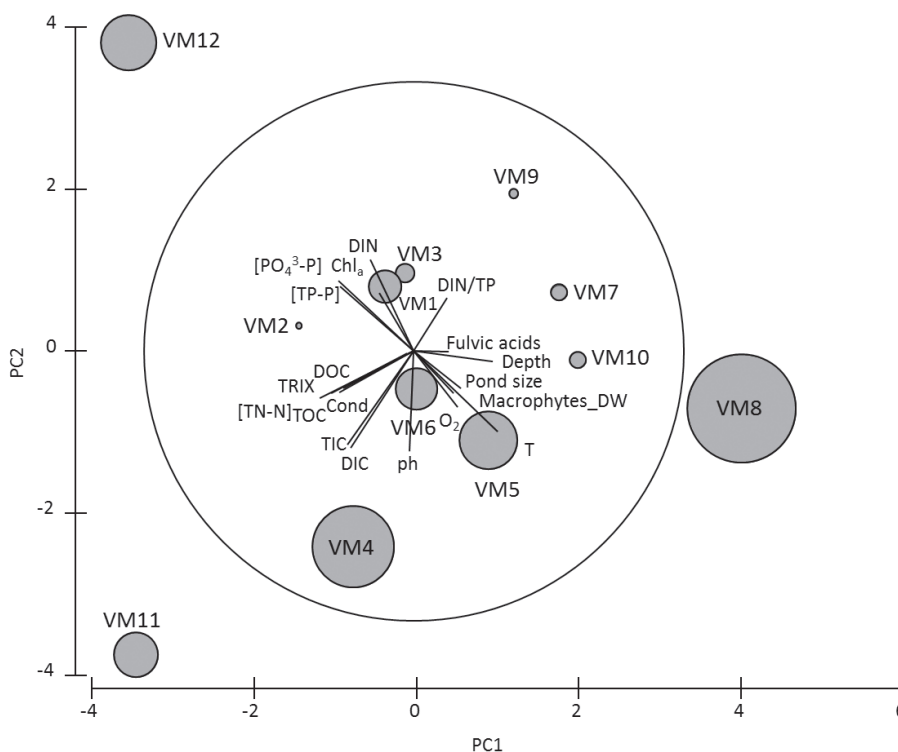


Figure 3. PCA plot showing the position of ponds in relation to the physical and chemical characteristics of the water. The size of the circles is proportional to pond size. The closer a variable is to the circle of correlations, the better it can be reconstructed from the first two components (and the more important it is to interpret these components); the closer a variable is to the centre of the plot, the less important it is for the first two components. The codes correspond to the sampled ponds (see Fig. 1). *Gráfico del PCA mostrando la posición de las lagunas en relación a las variables físicas y químicas del agua. El tamaño de los círculos es proporcional al tamaño de la laguna. Cuanto más cerca está la variable del círculo, mejor puede ser reconstruida a partir de los dos primeros componentes (y más importante es para interpretar esos componentes); cuanto más cerca está la variable del centro del gráfico, menos importante es para los dos primeros componentes. Los códigos corresponden a las lagunas muestreadas (ver Fig. 1).*

arose, neither for pond size (Fig. 5) nor for the rest of environmental factors tested (Table 2).

In analysing the relationship between pond size and the environmental variables that can po-

tentially modulate the pond size effect, no significant relationships were found (connectivity: $F_{1,10} = 3.3460$, $p = 0.097$; TRIX: $F_{1,10} = 0.0002$, $p = 0.99$ and macrophyte biomass: $F_{1,10} = 0.2383$,

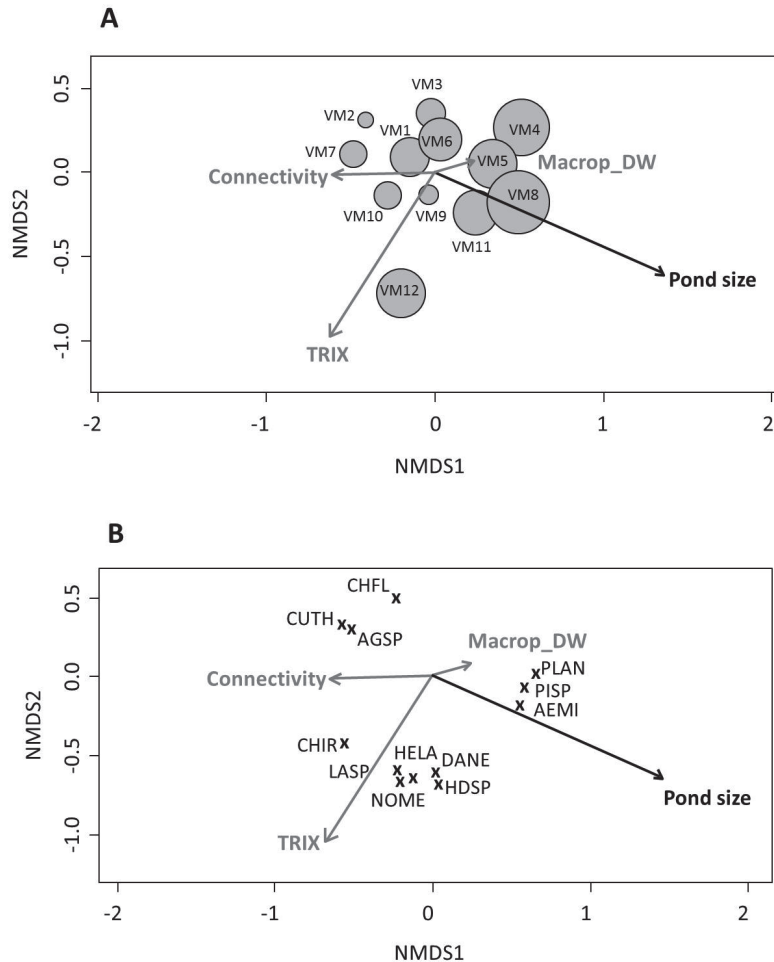


Figure 4. **A**) NMDS plot showing ponds identified by means of their code (see Fig. 1) according to their taxonomic composition. The size of the circles is proportional to pond size. Environmental factors (connectivity, TRIX, macrophyte biomass (Macrop_DW), and pond size (as the natural logarithm of pond size) are represented by arrows. The black arrow indicates the environmental variable with a significant effect ($p = 0.006$). Grey arrows represent non-significant variables. **B**) NMDS plot showing the taxa with a significant effect ($p \leq 0.05$) on the community composition. Acronyms stand for PLAN (*Gyraulus laevis*), PISP (*Piona* sp.), AEMI (*Aeshna mixta*), DANE (*Dicranomyia/Atypophthalmus/Neolimonia*), HDSP (*Hyphydrus* sp.), HELA (*Helophorus lapponicus*), NOME (*Notonecta meridionalis*), LASP (*Laccophilus* sp.), CHIR (Chironominae), AGSP (*Agabus* sp.), CUTH (*Culex theileri*) and CHFL (*Chaoborus flavicans*). **A**) Gráfico del NMDS mostrando las lagunas identificadas con su código correspondiente (ver Fig. 1) en función de su composición taxonómica. El tamaño de los círculos es proporcional al tamaño de la laguna. Los factores ambientales (conectividad, TRIX, biomasa de los macrófitos (Macrop_DW) y tamaño de la laguna (como el logaritmo neperiano del tamaño de la laguna) están representados con flechas. La flecha negra indica que la variable ambiental tiene un efecto significativo ($p = 0.006$). Las flechas grises representan variables no significativas. **B**) Gráfico del NMDS mostrando los taxones con un efecto significativo ($p \leq 0.05$) en la composición de la comunidad. Los acrónimos representan: PLAN (*Gyraulus laevis*), PISP (*Piona* sp.), AEMI (*Aeshna mixta*), DANE (*Dicranomyia/Atypophthalmus/Neolimonia*), HDSP (*Hyphydrus* sp.), HELA (*Helophorus lapponicus*), NOME (*Notonecta meridionalis*), LASP (*Laccophilus* sp.), CHIR (Chironominae), AGSP (*Agabus* sp.), CUTH (*Culex theileri*) and CHFL (*Chaoborus flavicans*).

$p = 0.636$). Therefore, no covariation exists between pond size and the environmental variables tested.

DISCUSSION

Pond size and community structure

Size has been noted on several occasions as being a determinant factor for the community composition of ponds (Rundle *et al.*, 2002; Vanschoenwinkel *et al.*, 2009). Similarly, in the case of lakes, several studies have shown an influence of the ecosystem size on community structure (e.g., Post *et al.*, 2000; S ndergaard *et al.*, 2005). Nevertheless, when we focus on ecosystems with higher environmental variability, such as Mediterranean temporary ponds, the influence of ecosystem size has been less documented. In our study, we did not find a strong effect of pond size on macrofauna, since size seems to influence the faunal composition but not structural parameters. Similarly, Rundle *et al.* (2002) found that the abundance of four coleopteran species was positively related to pond size. Thus, they found variation in the invertebrate composition,

which also occurred in our study. Considering our results, none of the community structure parameters had a significant relationship with pond size. Likewise, Gasc n *et al.* (2009) studied the relationships between six parameters that we also tested and the size of some temporary ponds, and they also found no significant relationships. Although Jeppesen *et al.* (2000) found an increase in the Shannon-Wiener diversity index for phytoplankton with lake area, we did not find a significant relationship between pond size and the Shannon-Wiener diversity index for macrofauna. Nevertheless, this lack of relationship is not rare, since some studies have found a significant positive relationship between habitat area (lakes or seas) and species richness (S ndergaard *et al.*, 2005; Azovsky, 2010), but many others were unable to find such relationships (Garc aValdecasas *et al.*, 1984; Friday, 1987; Jeffries, 1991). Moreover, Oertli *et al.* (2002), when working on permanent ponds, observed a positive relationship between area and the richness of some macroinvertebrate taxa such as Odonata and Gastropoda, but they found no significant relationships for Sphaeriidae, Coleoptera or Amphibia. In accordance with this, Della Bella *et al.* (2005) noted that the num-

Table 2. The statistics F and p are shown for the different linear regression models performed between community structure parameters (response variables) and environmental variables (explanatory variables). The fitness of the regression coefficients was not statistically different from the intercept-only model ($p > 0.05$). See Fig. 5 for acronyms. *Se muestran los estadísticos F y p para los diferentes modelos de regresión lineal llevados a cabo entre los parámetros de estructura de la comunidad (variables respuesta) y las variables ambientales (variables explicativas). El ajuste de los coeficientes de regresión no fue estadísticamente diferente del modelo de intersección única ($p > 0.05$). Mirar Fig. 5 para los acrónimos.*

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

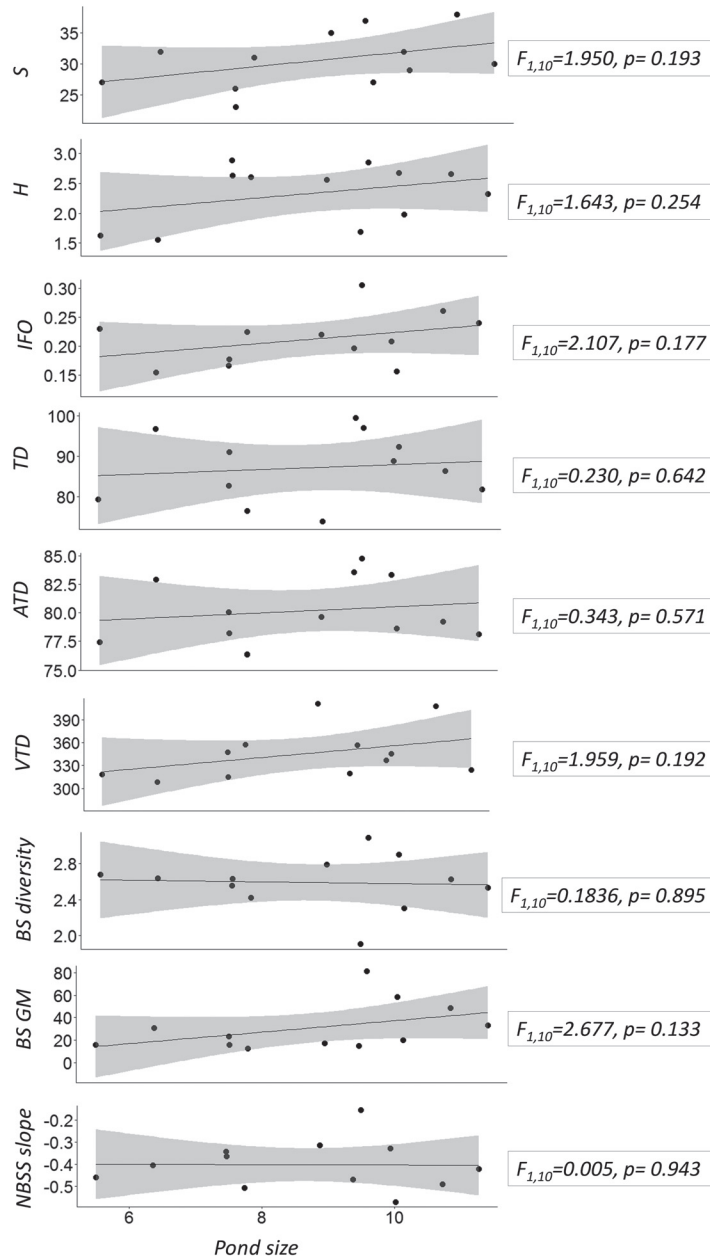


Figure 5. Relationship between pond size (natural logarithm of pond size) and the different community structure parameters. The results obtained by means of linear regression models are shown; the fitness of the regression coefficients was not statistically different from the intercept-only model ($p > 0.05$). Acronyms and symbols stand for S (richness), H' (Shannon-Wiener index), IFO (index of faunal originality), TD (taxonomic distinctness), ATD (average taxonomic distinctness), VTD (variation in taxonomic distinctness), BS diversity (body size diversity), BS GM (body size geometric mean), and NBSS slope (normalised biomass-size spectra slope). *Relación entre tamaño de la laguna (logaritmo neperiano del tamaño de la laguna) y los diferentes parámetros de estructura de la comunidad. Se muestran los resultados obtenidos con los modelos de regresión lineal; los ajustes de los coeficientes de regresión no fueron estadísticamente diferentes del modelo de intersección única ($p > 0.05$). Los acrónimos y símbolos representan: S (riqueza), H' (índice de Shannon-Wiener), IFO (Índice de Originalidad Faunística), TD (Diferenciación taxonómica), ATD (Media de la diferenciación taxonómica), VTD (Varianza de la diferenciación taxonómica), BS diversity (diversidad del tamaño corporal), BS GM (media geométrica del tamaño corporal), NBSS slope (pendiente de los espectros normalizados de biomasa-tamaño).*

ber of Coleoptera was not related to the pond size (including both permanent and temporary), presenting a different pattern in species richness from Odonata, Chironomidae and Hemiptera. In temporary environments, Spencer *et al.*, (1999) found that the species richness and predator-prey ratio were positively correlated with the maximum pool surface area, while Bilton *et al.* (2001) found no pond size effect on the species richness nor on the predator-prey ratio. The latter authors explained these contrasting results by the existence of a critical pond size, above which the detection by colonists is more likely to occur. In fact, we have noticed that the studies that found a positive relationship between pond size and species richness in temporary ponds (Ebert & Balko, 1984; March & Bass, 1995; Spencer *et al.*, 1999) included smaller water bodies than those in studies that did not find this relationship, such as the current study or the study by Bilton *et al.* (2001). Furthermore, some authors (e.g., Oertli *et al.*, 2002; Gaston & Spicer, 2004) have stated that most studies that found a positive relationship between area and richness did not remove the sampling effort effect. This does not seem to be the case in our study, since we removed the sampling effort effect during field sampling. In summary, our results reflect an unclear effect of pond size in temporary habitats because we found a significant relationship between pond size and community composition, but we found no relationship between pond size and any of the community structure parameters.

Other environmental drivers of community structure

The effect of pond size on community structure and composition can be modified by other factors such as habitat structure, water chemistry and connectivity. However, the indirect effects of pond size on community structure (i.e., larger ponds have different water physical and chemical characteristics than those that are smaller) seem to be weak in Mediterranean temporary ponds (Ballón *et al.*, 2016). Aquatic macrophytes play an important role in habitat structure and are highly influential in the composition of the

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

Using a metacommunity approach as a tool for conservation strategies

Metacommunities (Hanski & Gilpin, 1991; Wilson, 1992; Holyoak *et al.*, 2005) constitute a good theoretical framework to improve conservation strategies, allowing the inclusion of regional processes in management. In this sense, the pond network of Vila Nova de Milfontes

can be considered to be a metacommunity with high spatial connectivity within the “pondscape” (for information on the pondscape concept, see Baguette *et al.*, 2012). Thus, the exchange of a high proportion of species that characterises a metacommunity (Leibold *et al.*, 2004) makes it especially important to focus conservation strategies on the entire pond network and not only on a few ponds to maintain regional biodiversity. The absence of differences, for instance, in species richness and diversity among ponds of different sizes should be taken into account in conservation policy and management, since pond size has sometimes been used as a criterion to prioritise resources for conservation (Oertli *et al.*, 2002). Hence, it is important to promote the conservation of ponds regardless of their size. Small ponds can harbour the same species as bigger ponds, and the small ponds can act as stepping stones between larger ponds, helping to maintain metacommunity dynamics and stability (Leibold *et al.*, 2004; Vanschoenwinkel *et al.*, 2009). Thus, as has also been previously demonstrated, it is important to preserve pond networks because the spatial distribution of ponds influences pond quality as well as species distributions and dynamics (Gibbs, 2000; Jeffries, 2005).

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