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# Crustacean diversity and conservation value indexes in pond assessment: implications for rare and relict species

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**ABSTRACT**

**Crustacean diversity and conservation value indexes in pond assessment: implications for rare and relict species**

Ponds are important for the conservation of biodiversity. In order to safeguard them, it is essential to identify the habitats that sustain relict, rare or vulnerable species. This tactic has been utilized in communities of higher organisms but is rarely employed in pond crustacean communities, which are evaluated by species richness or diversity indexes in which all species are weighted equally. We have examined crustacean diversity in relation to environmental gradients in 150 Mediterranean ponds and propose a crustacean conservation value index (CVVI) for ponds. Under this index, species are scored based on the relictness and biogeographical significance of the taxonomic group to which they belong, as well as on their rarity or endemicity. In our study, permanent ponds showed a higher accumulated number of crustacean species than temporary ponds; however, local species richness was not consistently higher in permanent ponds, and the highest species richness was in fact found in temporary ponds. However, when ponds were subdivided by type into three groups of temporary ponds and two groups of permanent ones, species richness and diversity parameters tended to increase with an increasing degree of stability in both temporary and permanent ponds. In contrast, the accumulated CCVI was more than three times higher for groups of temporary ponds. A comparison of CCVIs revealed the importance and singularity of temporary pond fauna, indicating that species richness must not be the only criterion used to prioritize the conservation of habitats. Within a homogeneous pond typology, species richness could be a useful parameter for identifying priority habitats; however, the conservation value index per species includes other aspects of biodiversity more suitable to preserve locations with ancestral or original regional fauna. These fauna are currently endangered and should be prioritized for conservation, as increasing human activity favors the opportunistic and invasive species that are displacing them.

**Key words:** Mediterranean Temporary Ponds, species richness, management, priority habitats.

***RESUMEN***

***Diversidad y valor indicador de las especies de crustáceos para la conservación de las charcas: implicación de las especies raras y relictas***

*Las charcas y pequeños ambientes acuáticos juegan un papel importante en la conservación de la biodiversidad y para salvaguardar las más valiosas es fundamental reconocer los hábitats que albergan especies relictas, poco comunes o en riesgo de extinción. Esto se valora normalmente en los organismos superiores, pero apenas se ha tenido en cuenta en las comunidades de crustáceos de las charcas que se evalúan por la riqueza de especies o la diversidad, considerando todas las especies por igual. En este trabajo hemos analizado los patrones de diversidad de crustáceos en diversos tipos de charcas mediterráneas en relación con gradientes hidrológicos y medioambientales con las aproximaciones clásicas, pero además proponemos un nuevo índice dirigido a evaluar la conservación de estos hábitats basado en la importancia biogeográfica y en la rareza de los crustáceos presentes (CCVI, Crustacean Conservation Value Index). En nuestro estudio de 150 charcas hemos observado que, aunque el grupo de charcas permanentes presenta un número total mayor de especies de crustáceos que las temporales, a escala local las diferencias no son tan claras, y algunas charcas temporales presentaron los valores más altos de riqueza de especies. Sin embargo, cuando se comparan cinco tipologías de charcas (3 tipos de charcas temporales y 2 de permanentes) en un gradiente de menor a mayor hidroperiodo y condiciones ambientales más estables, observamos que tanto la riqueza de especies como la diversidad y otros parámetros relacionados, sí que aumentan con el grado de estabilidad propio del tipo de charca, tanto dentro de las temporales como de las permanentes. Por otro lado nuestros resultados ponen* *de manifiesto la importante contribución de índices basados en el interés biogeográfico y la rareza de las especies, en la identificación de los lugares prioritarios para la conservación de la biodiversidad. El índice propuesto utilizado en este estudio pone de relieve el interés y singularidad de ciertas charcas temporales muy efímeras, con pocas especies tal vez, pero claves en la conservación de especies con alto riesgo de extinción. Las charcas temporales alcanzan un CCVI acumulado tres veces superior a las permanentes. La riqueza de especies puede ser útil como métrica para identificar hábitats prioritarios o definir un mejor estado ecológico dentro de hábitats con un tipo similar de comunidad, sin embargo el índice de valor para la conservación (CCVI) incluye otros aspectos de la biodiversidad relevantes para conservar aquellos hábitats que albergan la fauna ancestral u originaria del lugar. Esta fauna es la que presenta ahora más riesgo de extinción y debe tener más puntuación en los índices usados para determinar las áreas prioritarias para la conservación, ya que el incremento de las actividades humanas favorece cada vez más las especies oportunistas o invasoras que las están desplazando.*

***Palabras clave:*** *Lagunas temporales Mediterráneas, riqueza de especies, gestión, hábitats prioritarios.*

## INTRODUCTION

Ponds are highly abundant and varied aquatic habitats that are found throughout the world (Downing *et al.*, 2006). Recently, they have attracted the attention of conservationists and researchers due to their disproportionate contribution to biodiversity as a whole (Oertli *et al.*, 2002; Williams *et al.*, 2004) and their utility as model systems for hypothesis testing (De Meester *et al.*, 2005). Despite their importance, ponds are highly sensitive and threatened aquatic habitats that remain poorly known (Beja & Alcazar, 2003; Dimitriou *et al.*, 2006; Grillas *et al.*, 2004; Serrano & Esquivias-Segura, 2008; Zacharias *et al.*, 2007). In Mediterranean regions, ponds hold special value due to the scarcity of water, not only because they provide water for traditional uses but also because of the presence of a periodic dry phase that leads to a unique biota (Williams, 2006; Díaz-Paniagua *et al.*, 2010; Bagella *et al.*, 2010; Marrone *et al.*, 2006; Alvarez Cobelas *et al.*, 2005). Furthermore, the Mediterranean region served as a refuge during the climatic fluctuations of the ice age and currently preserves a very specific freshwater fauna of typical Mediterranean steppe taxa in the drier regions and Central European elements in wetter areas (Miracle, 1982; Marrone *et al.*, 2010). However, ponds span a wide range of environmental characteristics from permanent ponds fed by groundwater to ephemeral rain-fed ponds, even in semi-arid countries. In fact, the high biodiversity of ponds is caused by the wide range of environmental characteristics that they supply (Davies *et al.*, 2008; Williams *et al.*, 2004).

The importance of ponds for biodiversity is undisputed, and several pond-associated habitats and species are protected under international laws such as the Habitats Directive (Council Directive 92/43/EEC). However, it is rare that specific protective plans for these habitats are in place. The large number of ponds, combined with their small size and their scattered distribution, makes it necessary to prioritize areas for protection. The characterization of biodiversity may assist these conservation projects such as, for example, the Important Areas for Ponds Project (IAPs), which was set up to identify Priority Ponds using standardized biological criteria (Ewald *et al.*, 2010). Scoring methods for assessing the value of a single pond or area based on biodiversity may follow two approaches: (1) quantifying the number of species present or the distribution of individuals in species, while giving all species the same weight; and (2) assigning a value to each species to identify its "faunal quality" (e.g., Red List species). Both metrics must be taken into account when assessing a habitat's biodiversity (Bo*ix et al.*, 2005; Colli*nson et al.*, 1995). In fact, ponds are both biodiversity hotspots and critical habitats of uncommon or rare species (EPCN, 2008).

In previous work (Sahuquillo & Miracle, 2013), we identified 5 different pond types among a set of 150 Mediterranean ponds in the

Eastern Iberian Peninsula based on their crustacean communities. In the present paper, we will assess differences in diversity parameters among these pond types in order to look for indexes useful for determining their conservation value. We will compare patterns with known indexes to estimate species richness or diversity, as well as rarity, uniqueness or singularity among different pond types. In addition, we propose an index of pond conservation value based on the biogeography and rarity of crustacean species. This kind of index, known as a Conservation Value Index (CVI), has been successfully applied to other biological groups (plants, amphibians or macroinvertebrates) (Rosset *et al.*, 2012, Eyre & Rushton, 1989; Foster *et al.*, 1992; Linton & Goulder, 2000; Oertli *et al.*, 2002; Painter, 1999; Williams, 2000). Here, we propose criteria for adapting the CVI to pond-dwelling crustaceans (results obtained from groups which have a terrestrial phase are not applicable to crustaceans). Among other invertebrates, crustacean zooplankton, which are permanent inhabitants of aquatic systems, are valid indicators of the ecological conditions of water bodies. They are important freshwater fauna and are particularly significant in the food webs of stagnant waters. Moreover, the crustacean community has been used previously as an indicator of ecological status –or "health"– of the aquatic ecosystem (Boix *et al.*, 2005; Camacho *et al.*, 2009; Moss *et al.*, 2003). Some temporary ponds support unique crustacean species, such as large branchiopods which may be a suitable group for the application of this index in this kind of ponds. Using crustacean data from a wide variety of ponds in Mediterranean Spain, we investigate whether metrics based on species richness or on "faunistic quality" provide similar or complementary information regarding the relative conservation value of ponds. In light of the fast pace at which ponds are destroyed or deteriorated, our aim in this paper is to contribute useful tools for establishing effective conservation strategies for pond biological biodiversity. Namely, we sought to improve tools for the recognition of threatened and special habitats to prioritize during conservation efforts whose loss would represent the irreversible disappearance of relict or specialised singular species.

## METHODS

### The study area

The study area –Comunitat Valenciana– is located on the central Mediterranean coast of the Iberian Peninsula and covers a strip of 500 km of coastline from North to South and 100 km westward from the coast. The inland part of the territory is mountainous and outlines large sedimentary lowland coastal plains. The region is characterized by a Mediterranean climate, with pronounced spatial variation (Pérez Cueva, 1994) ranging from semiarid (mean annual rainfall: 300 mm/year) to sub-humid (above 800 mm/year) zones created by continental influence and mountain orientation. Ponds are frequent in the study area (Sancho & Lacomba, 2010) and display diverse environmental characteristics. Most water bodies are small and shallow. The area of 50 % of the studied ponds was $< 200$ m$^2$ (only 4 % $> 2000$ m$^2$) with depths varying from 0.4 to 4 m. These ponds cover a broad range of hydroperiods, from ephemeral rain-fed ponds to permanent ponds fed by groundwater. Thus, they constitute an appropriate dataset for investigating the influence of graded hydrological variation on pond limnology and faunal composition. We have thus carried out an extensive survey of ponds in this area to investigate several limnological features. A description of the physical and chemical characteristics of these ponds can be found in Sahuquillo *et al.* (2012), which focused on nutrient relationships, as well as planktonic chlorophyll, clay turbidity and plant cover. A pond typology based on crustacean communities has been reported by Sahuquillo & Miracle (2013). These studies document the influence of the hydroperiod in shaping the characteristics and community structure of the ponds. In other studies, the biodiversity of large branchiopod fauna has been noted Miracle *et al.*, 2008), as has been crustacean community succession in a unique relict pond (Sahuquillo & Miracle, 2010).

## Sampling

Crustaceans were sampled from 150 ponds unevenly distributed in the study area to cover different geographic and climatic zones. These ponds were grouped into the following five pond types, according to their distinct crustacean communities (Sahuquillo & Miracle, 2013): (I) temporary ponds in arid areas with a mean annual rainfall below 600 mm (46 ponds, 74 samples); (II) temporary ponds in wetter areas (38 ponds, 46 samples); (III) special temporary ponds with a unique crustacean community-termed "*Hemidiaptomus* ponds" due to the presence of this genus of calanoid copepods (Sahuquillo & Miracle, 2013) (3 ponds, 33 samples); (IV) mountain permanent ponds (52 ponds, 53 samples); and (V) lowland permanent ponds fed by subterranean springs (11 ponds, 40 samples). Samples were collected mainly from February to May in 2006 and 2007 in different but partially overlapping sets of ponds such that half of the ponds were sampled in both years (a few ponds were resampled in spring 2008). Permanent lowland ponds were sampled in late winter, early summer and autumn, and special temporary ponds were sampled several times during their ponding phase (winter-spring and autumn in some years), as were a few ponds from the other groups of temporary ponds. Thus, for most ponds, we have data from one or two sampling campaigns, but for less frequent pond types, we used data from several campaigns. We assume that our sampling program, based on a large number of "common" ponds studied over a large area, compensates for the low frequency of sampling. We also assume that the higher sampling frequency compensates for the low number of "special and singular" ponds studied because the turnover of species in space and time is not independent (Fridley *et al*., 2006).

Because ponds are small, it is relatively easy to catalog the species that they support. Nevertheless, as different sampling efforts and methodologies can bias richness parameters, we took special care to include maximum spatial diversification by sampling different microhabitats. For each pond and sampling date, we obtained a "combined sample," which we will refer to simply as a "sample." Littoral and plant-associated crustaceans were collected by sweeping a 90 μm hand-net through vegetated areas and shallow shores, and open water crustaceans were collected by towing 45 μm and 250 μm nets across the entire pond and also with a transparent 0.5 m or 1 m long cylinder through the water column. Hayek & Buzas (1997) suggest that a total of 200-500 individuals is adequate for estimating diversity, while other authors (Sørensen *et al*., 2002) have proposed that at least 30-50 specimens per major species should be counted. In average, we counted

**Table 1.** Criteria used to assign a conservation score to each crustacean species. *Criterios utilizados para asignar una puntuación a cada especie de crustáceo*.

| For Cladocerans and Cyclopoids | |
|---|---|
| **Score** | **Criteria** |
| 1 | Common Cladoceran or Cyclopoid |
| 2 | Local crustaceans either confined to certain limited geographical areas (e.g. Circum-Mediterranean), where populations may be common, or with a more widespread distribution, but holding few populations. |
| 4 | Rare species with few populations |
| 8 | Endemism recorded only from Iberian Peninsula or from very restricted areas |
| **For Calanoids and Large Branchiopods** | |
| **Score** | **Criteria** |
| 4 | Widespread calanoid |
| 8 | Restricted calanoid or common anostracan |
| 16 | Rare anostracan |
| 32 | Notostracan, endemic anostracan and very restricted calanoid |
| 64 | Spinicaudatan |

more than 800 individuals (including nauplii, juveniles and adults) per visit, with a mean of 60 individuals per species in lowland permanent ponds, 309 in other permanent ponds, and over 800 in temporary ponds. These ratios reflect the different structures of zooplankton assemblages among the sites, but are independent of sample size. More information about sampling and a complete taxonomic list of crustaceans recorded in the 150 ponds is available in Sahuquillo & Miracle (2013).

### Indexes and estimators

In order to compare biodiversity patterns, we calculated the following indexes and estimators and applied them on different scales: local (for a given pond and sampling date) and accumulated (for a given pond type).

1. **Species richness** was estimated as the total number of crustacean species occurring in a site or in a pond type. Because the number of samples collected influences richness, we standardized this value in a scale-dependent manner. The **local species richness** –or point species richness (Magurran, 2004)– was calculated as the total number of crustacean species recorded in each pond at each date. Sampling was designed to maximize the number of species detected, but for the sake of comparison, we also standardized local species richness upon rarefaction to 100 individuals using Primer 5 software. The **accumulated species richness per pond type** is the total number of species encountered in all samples collected from each pond type. To standardize this parameter, we estimated total species richness with sample-based rarefaction curves (**cumulative** Mao Tau) and **asymptotic richness** (MMMeans). We also calculated two additional estimators of total species richness: the bias-corrected **Chao2 index** and the resampling method **Jackknife2**. Sample rarefaction curves and richness estimators were computed using EstimateS (Version 7.5, R. K. Colwell, 2004, http://purl.oclc.org/estimates). In addition, the following indexes were computed as a measure of heterogeneity within each pond type: the **Whittaker index** (the ratio of accumulated species richness and mean local species richness) and Uniques and Duplicates (**Q1** and **Q2**; the number of species that occur, respectively, in only one sample or in only two samples among all samples of each pond type).
2. **Diversity index:** We calculated Shannon index diversity and Pielou evenness using the statistical program PRIMER 5 V5.2 (PRIMER-E Ltd.) and the corresponding **effective number of species** [exp $(H')$].
3. **Singularity indexes: Uniqueness** of pond type was calculated for each pond type as $U = (s/S) * 100S$, where $s$ = the number of species found in a given type of ponds and not in any other pond type and $S$ = the total number of species found in that pond type. **Index of faunal originality** (IFO, Puchalski, 1987) was calculated for each pond as IFO = $(\sum 1/M_i)/S$, where $M_i$ = the total number of ponds of a single pond type in which species i occurs and $S$ = the total number of species in the corresponding pond.
4. To further evaluate the **Conservation Value** of each pond, we used a Crustacean Conservation Value Index (CCVI) to account for the biogeography, relictness, degree of rarity and risk of extinction for each crustacean species. This index was derived following a procedure that has been commonly used for other groups of organisms (i.e., Oertli *et al.*, 2002): (I) all species present are scored according to their rarity, vulnerability or threat; (II) the scores of all species in each sample were summed to give a Species Rarity Score; and (III) the Species Rarity Score was divided by the number of species recorded in the sample to yield the CCVI. Due to a lack of Red Lists for microcrustaceans, we attempted to assign a conservation score to each crustacean species found based on the relictness and biogeographical significance of its main taxonomic group and its

endemicity or rareness in the studied ponds. Scores were scaled with powers of two according to criteria defined in Table 1.

## RESULTS

We first compared the richness, diversity and singularity between temporary and permanent ponds (i.e., pond types I, II and III vs. pond types IV and V). We then studied differences between the aforementioned pond types. These pond types were then ranked by increasing habitat stability from type I to V: (I) rain-fed ponds in **arid** areas, where rainfall is scarce and unpredictable, (II) temporary ponds located in **wetter** areas with longer and more predictable hydroperiods (III) a small group of relict temporary ponds containing **special** fauna (*Hemidiaptomus* ponds) suggestive of their ancient origin and good state of preservation. (IV) permanent or semipermanent ponds in **mountains** associated primarily with temporary streams or springs in mountain slo-

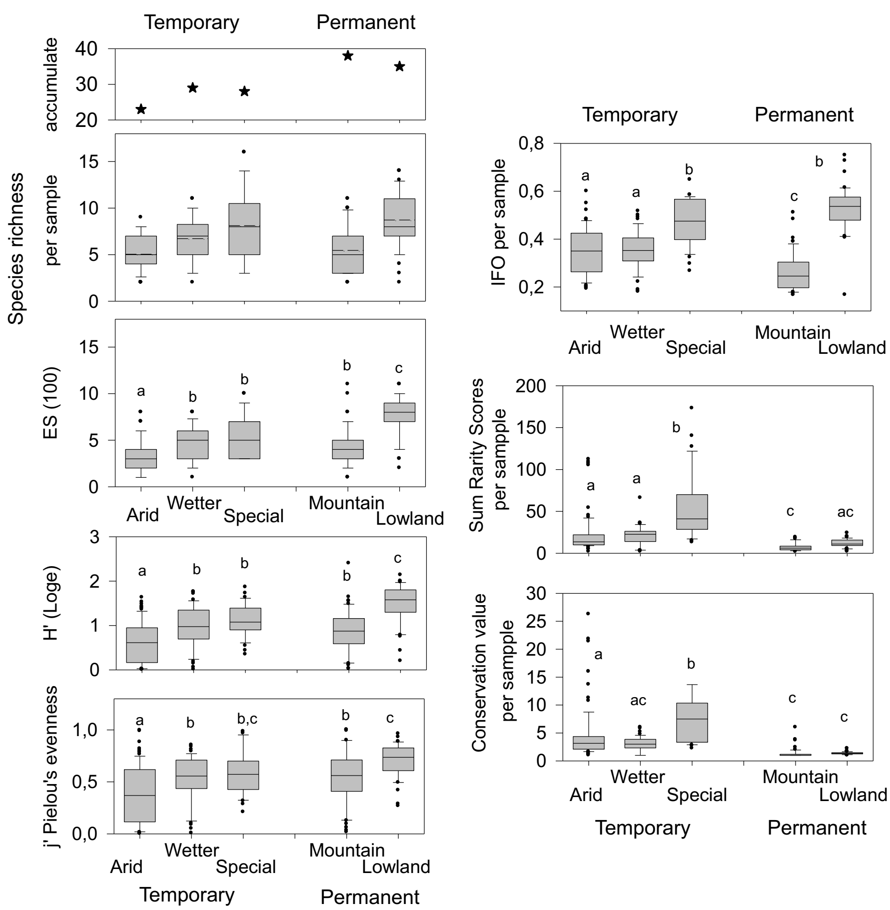



**Figure 1.** Boxplots showing several diversity estimators for each pond type: (I) Arid, (II) Wetter, (III) Special, (IV) Mountain, (V) Lowland. Local species richness was calculated for individual samples (i.e., per pond and sampling date) and for the accumulated richness of pooled samples within each pond type. The Shannon diversity index ($H'$), Pielou evenness, IFO rarity score and crustacean conservation value index (CCVI) were calculated for individual samples. Different letters over the box-plots refer to significant differences among pairwise comparisons determined by Tukey post hoc tests. *Diagramas de caja mostrando los resultados para los diferentes tipos de charcas. La riqueza local de especies se ha calculado por muestra (por charca y día de muestreo) y la acumulada en el total de las muestras de cada tipología. Los índices de diversidad de Shannon (*H'*), la equitabilidad de Pielou, la rareza (IFO) y el valor para la conservación de los crustáceos (CCVI) se calcularon por muestra. Las letras sobre los gráficos indican los grupos significativamente diferentes (pruebas post-hoc de Tukey).*

pes, some of them remodelled for irrigation and (V) permanent ponds in **lowlands** fed by groundwater (spring pools), with less fluctuation in water level, stable temperatures throughout the year, continuously flowing waters, and the presence of fish.

## 1. Species richness

At a **local** scale, the average number of species observed on a single sampling date in temporary ponds was not significantly different than in permanent ponds (Table 2). When rarefaction over 100 individuals was considered, species richness appeared lower in both cases, but became significantly higher in permanent ponds. However, the highest local crustacean species richness was found in a temporary pond (Lavajo de Abajo, type III), in which 16 species were observed on several occasions in both spring and autumn. But when taking into account the **accumulated** number of species in these two kinds of ponds, temporary ponds supported fewer crustacean species than permanent ponds (Table 2).

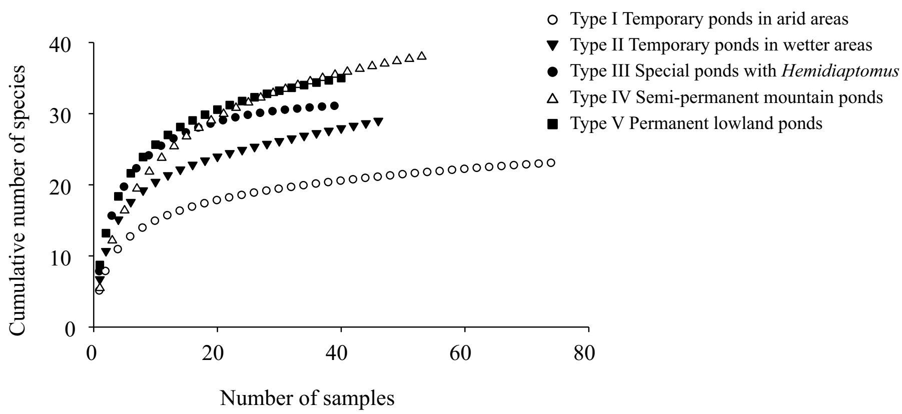
Differences in species richness become clearer upon comparing this parameter among the five pond types ordered along a gradient of wa-

**Table 2.** Comparison of crustacean species richness, diversity, rarity and CCVI between temporary and permanent ponds, and the results of ANOVA ($p$) with log-transformed variables. In bold: significant results. *Comparación de la riqueza de especies, diversidad, rareza y CCVI entre las charcas temporales y las permanentes, y resultados de ANOVA (*p*) con las variables transformadas logaritmicamente. En negrita, los resultados significativos.*

| | TEMPORARY | | PERMANENT | | |
|---|---|---|---|---|---|
| Nº ponds | 88 | | 63 | | |
| Nº samples | 154 | | 93 | | |
| **1. Species richness** | | | | | |
| | Accum | Local Mean ± SE (max) | Accum | Local Mean ± SE (max) | $p$ |
| Sp. richness | **45** | 6.21 ± 2.8 (16) | 57 | 6.9 ± 3.2 (14) | 0.135 |
| ES (100) Rarefaction 100 | | **4.11 ± 2.1** (10) | | **5.68** ± 2.7 (11) | 0.000 |
| Nº Large Branchiopod | **8** | **0.56 ± 0.8** (0-4) | **0** | 0 | 0.000 |
| Nº Planktonic Cladocera | **8** | **1.53 ± 1** (4) | **11** | **0.66 ± 0.8** (2) | 0.000 |
| Nº Planktonic Copepoda | **15** | **1.92 ± 0.9** (5) | **12** | **1.61 ± 0.9** (4) | 0.002 |
| Nº Littoral Cladocera | **13** | **1.95 ± 1.7** (7) | **22** | **2.58** ± 1.6 (7) | 0.001 |
| Nº Littoral Copepoda | **3** | **0.26 ± 0.5** (2) | **13** | **2.00** ± 1.6 (7) | 0.000 |
| **2. Diversity indices and Singularity** | | | | | |
| $H'$ (Shannon diversity). log $e$ | | **0.82** ± 0.5 (1.86) | | **1.15** ± 0.6 (2.4) | 0.000 |
| Nº sps uniques | 21 | | 32 | | |
| Uniqueness | 48 | | 58 | | |
| IFO | 0.56 | 0.38 ± 0.1 (0.18-0.65) | 0.65 | 0.38 ± 0.1 (0.17-0.75) | 0.849 |
| **3. Crustacean Conservation Value** | | | | | |
| Sum Rarity Scores | | **25** ± 1.9 (173) | | **9.3** ± 0.5 (24) | 0.000 |
| CVI | | **4.6** ± 0.3 (26) | | **1.35** ± 0.1 (6) | 0.000 |

ter permanency and stability (gradient of harshness). Local species richness increased (Fig. 1) as pond types were more stable and predictable. The **accumulated** species richness increased within temporary ponds under more benign conditions (from type I to III), but mountainous semi- and permanent ponds had the highest value within permanent ponds, indicating that it is a more heterogeneous group (Fig. 2). This was also confirmed by the Whitaker index and by the number of species occurring only in 1 or 2 samples (Q1+Q2).

Sample rarefaction curves provided additional information on the accuracy with which the analysed samples reflect true biodiversity. The curves representing the number of species




| Pond Types | I Temporary Arid | II Temporary Wetter | III Temporary Special | IV Permanent Mountain | V Permanent Lowland |
|---|---|---|---|---|---|
| **1) Richness** | | | | | |
| Sobs Mau Tau (95%CI) | 23 (20-26) | 29 (22-36) | 28 (26-30) | 38 (34-42) | 35 (31-39) |
| MMMeans (increasing %) | 23 (0%) | 30 (3%) | 31 (11%) | 42 (11%) | 37 (6%) |
| Chao 2 | 25 (9%) | 56 (108%) | 29 (4%) | 42 (11%) | 40 (14%) |
| Jackknife 2 | 28 (22%) | 45 (67%) | 31 (11%) | 48 (26%) | 43 (23%) |
| Q1 + Q2 | 7 | 8 | 6 | 14 | 10 |
| Whittaker index | 5 | 4 | 3 | 7 | 4 |
| **2) Maximum diversity per pond and sampling date** | | | | | |
| Shannon Diversity Index Maximum | 1.6 | 1.8 | 1.9 | 2.4 | 2.1 |
| Effective number of species Maximum Exp H | 5.1 | 5.8 | 6.5 | 11.0 | 8.5 |
| **3) Median CCV per pond and sampling date** | | | | | |
| *Per* assemblage (Sum of species scores) | 13.5 | 22.5 | 41.0 | 1.0 | 1.4 |
| *Per* species CCVI | 3.2 | 3.0 | 7.5 | 2.4 | 2.1 |

**Figure 2.** Sample-based rarefaction curves of crustacean species richness ($S_{MaoTau}$ for the 5 different pond types). Each table contains the following for each pond type: 1) several estimates of accumulated species richness, 2) maximum sample diversity and effective number of species, and 3) median sample Crustacean Conservation Values per assemblage and per species (CCVI). Pond types are ordered from less to more stable. *Curvas de rarefacción de la riqueza especies de crustáceos ($S_{MaoTau}$) para los 5 tipos de charcas. En la Tabla, para cada tipo de charcas: 1) varios descriptores de la riqueza de especies acumulada, 2) diversidad y número efectivo de especies máximos y la mediana de los valores de conservación, como suma total de las puntuaciones y media por especie (CCVI). Los tipos de charcas están ordenados de mayor a menor estabilidad de las condiciones ambientales.*

*vs* samples (Fig. 2) reached an asymptote for temporary ponds in dry areas (Type I), for special ponds (Type III) and for lowland permanent ponds (Type V); thus, crustacean richness was adequately evaluated. However, we have to take into account that special ponds (Type III) were represented by only three sites, although each was sampled a large number of times. In contrast, in temporary ponds in wetter areas (II) and semi-permanent and permanent ponds in the mountains (IV), the cumulative species richness curves did not plateau.

### 2. Diversity

The average local diversity index was significantly higher for samples collected from permanent ponds than temporary ones (Table 2). The diversity index also highlighted important differences across the hydroperiod gradient, with an increasing trend towards more stable conditions (Fig. 1). Temporary ponds in arid areas (Type I) presented lower diversity and evenness values. Temporary ponds in wetter areas (Type II), special temporary ponds (Type III), and mountain permanent ponds (Type IV) yielded higher values, while lowland permanent ponds (Type V) presented the highest values.

### 3. Rarity and singularity

The accumulated singularity index (IFO) was higher in permanent than in temporary ponds, although the difference in local richness between the two groups of ponds was not statistically significant. Uniqueness was also higher for the group of permanent ponds (Table 2).

Taking into account the five pond types, IFO indexes resulted in important differences along the hydroperiod gradient, with an increasing trend towards more stable conditions (Fig. 1). Special temporary ponds (Type III) and lowland permanent ponds (Type V) supported more uncommon species (i.e., had a higher IFO) than other types. However, in contrast with its high within-group heterogeneity, the semi-permanent and permanent mountain pond type (Type IV) showed a significantly lower IFO.

### 4. Conservation value

In contrast with the previous indexes, both the sum of conservation scores and their average (CCVI) were approximately three times higher in temporary than in permanent ponds (Table 2). The CCVIs of temporary ponds varied from 1 to 26 and were significantly higher than those of permanent ponds (from 1 to 6).

Significant differences were also observed between the sums of rarity scores within each pond type (Fig. 1). However, differences were slightly smaller than between CCVIs, due to the higher species richness of permanent ponds. CCVI scores varied from 1 (i.e., all species are shared) to 26. This maximum value was obtained for a large ephemeral Type I pond (called Rebalsador) with an extremely short hydroperiod. This pond supports a low number of species, but they are very rare, being three groups of large branchiopods represented (anostracans, spinicaudatans and notostracans). This indicates the complementarity of the diversity and conservation value indexes. The second highest CCVI of 13 was obtained for a *Hemidiaptomus* Type III (special) pond (Lavajo de Abajo, Sinarcas). In this case, score can be attributed to both the high species richness of this pond and the singularity of its species.

## DISCUSSION

### Temporary *vs.* permanent ponds

From our results, we cannot conclude that there is a significant difference in local species richness between temporary and permanent ponds. The results of other studies are variable; some authors found that water permanence was an important predictor of crustacean species richness, with higher richness in permanent ponds (Ebert & Balko, 1987; Eitam *et al.*, 2004). On the other hand, Frisch *et al.*, (2006) reported higher species richness in temporary ponds with an intermediate hydroperiod, whereas Boix *et al.* (2008) did not detect significant differences. Our results support the latter study. Indeed, the mean and the maximum number of species found in each pond at each sampling date in our study was extremely similar to that reported by Boix *et al.* (2008) in an extensive study of ponds similar in size to those included in our study in an adjacent region of the Mediterranean Iberian Peninsula (Catalonia). Boix *et al.* (2008) reported that the species number ranged from 1-15 in temporary ponds and 1-13 in permanent ponds (we found 2-16 in temporary and 2-14 in permanent ponds). These numbers could serve as a guideline for the number of species expected to co-occur in Mediterranean ponds of a relatively small size. On the other hand, when we consider a larger scale (i.e., the accumulated species richness *per* pond group), permanent ponds show a richer pool of species than temporary ones. Although more major taxonomic groups are found in temporary ponds, they are usually represented by only a small number of specialized species (Bratton, 1990; Williams, 2006; Zacharias *et al.*, 2007). In other words, the environmental filter in temporary ponds is harsher than in permanent habitats (Chase, 2003). Thus, fewer species from the regional species pool can live in temporary habitats compared to permanent habitats. Consequently we found a similar community composition among temporary ponds, which may have high local species richness. Given that local species richness, regulated by interspecific interactions, should lead to some equilibrium value, the higher accumulated species richness in permanent ponds implies a higher degree of dissimilarity in their community composition. Similar results were obtained by Chase (2003), who reported that multiple stable equilibria are more common in local habitats with relatively benign environmental conditions (i.e., permanent ponds), whereas a single, recurrent equilibrium is more common under harsh environmental conditions. In the latter case, adaptation to the environment is the main driver of site-to-site variation in community composition. In the former case, different invasion sequences of euryecious species could also determine community composition, depending on the order of species colonization. Accordingly, permanent ponds will be more dissimilar from each other, and their accumulated species richness will be high. The dependence of species richness on the scale of observation has also been observed on a temporal basis. Serrano & Fahd (2005) studied temporary ponds in Doñana (South Iberian Peninsula) with different hydroperiods and showed that richness varied weakly across the hydroperiod gradient in the short term, but became strongly differentiated over a longer period of time.

With respect to the number of species belonging to major taxonomic groups, we found marked differences between temporary and permanent ponds. Microcrustacean groups whose species are well adapted to periods of drought and are easily preyed on by fish, such as large branchiopods and calanoid copepods, were restricted to or enriched in temporary ponds. Conversely, the number of species of cyclopoids and harpacticoids was higher in permanent ponds.

In the samples collected for this study, crustacean fauna were quite well characterized for temporary ponds, with 24 of the total 47 taxa found restricted to those ponds. This is comparable with findings reported for other Mediterranean areas such as Doñana (Fahd *et al.*, 2009) or Sicily (Marrone *et al.*, 2009). In our study, comparison of the community composition between temporary and permanent ponds revealed that a third of the observed species are present in both permanent and temporary waters. Moreover, when pond type is considered, a continuum can be drawn between temporary and permanent freshwater habitats, where the percentage of obligate species restricted to temporary habitats decreases with increasing hydroperiod. These results agree with the predicted changes in community composition along a temporary-permanent habitat gradient (Williams, 2006).

## Differences across a gradient of environmental stability

The lack of a clear difference in crustacean richness between temporary and permanent ponds is better understood when the results of our pond type analysis are considered. Local species richness tended to increase with increasing habitat stability, both in temporary and in permanent ponds. Within temporary ponds, we observed an increase in local and accumulated species with increasing habitat stability. In this sense, special ponds (including *Hemidiaptomus*, pond type III) stood out for the richness and singularity of crustacean species found. These ponds hold 35 % of the total number of crustacean species found in all ponds, and at least nine species were restricted to them (11 % of the total number of species). Additional restricted species could have gone undetected, because some species named according to today's taxonomy could in fact be undescribed cryptic species of a congeneric complex (Sinev *et al.*, 2012; Bode *et al.*, 2010; Korn *et al.*, 2010; Marrone *et al.*, 2013). This small set of temporary ponds presented a rich community that has likely adapted over their long history. Ongoing geological studies suggest that these ponds are located in a relict Pliocene area that was unaffected by Quaternary erosion (Santiesteban *pers. comm.*). The long-term stability of these habitats has likely provided a refuge for the survival of highly specialized crustaceans over time. These ponds are partially maintained by the water table, and their biodiversity may be explained by the "intermediate disturbance hypothesis" (Connell, 1978). On the other hand, temporary ponds located in more arid areas are susceptible to unpredictable flooding and drought (exceedingly high rates of disturbance), thus potentially restricting the number of species that can successfully develop in these habitats. In addition, coexisting species have adaptive faster development rates that would allow them to escape from dryness, thereby lowering diversity indexes.

The relation between local and accumulated species richness has several implications (Chase, 2003), as we mentioned above. The lower number of species adapted to less stable environments leads to a low accumulated species richness, as illustrated by the low values of temporary ponds in arid areas (Type I, Fig. 1 and 2). In the special ponds (Type III), local but not regional richness was high, because compositional differences between samples are reduced due to the environmental conditions that allow the survival of a relict assemblage of species and also to the small number of currently existing ponds of this type. Thus, despite their higher local diversity, the accumulated richness of Type III is similar to that found in temporary ponds in wet areas (Type II). Similar reasoning can be applied to permanent pond types. Lowland spring ponds represent more stable habitats than spring ponds in mountainous areas; they rarely fluctuate in both quality and quantity of water. In our study, we observed a low local richness but a high accumulated richness in semi-permanent/permanent ponds in mountains (Type IV). Dissimilarity between samples is straightforward (Fig. 1 and 2). This might be ascribed to the afore mentioned hypothesis of multiple stable equilibria (Chase, 2003); that is, differential colonization by equally fit species in similar habitats can cause a divergence in species composition. However, sites might not be environmentally similar, and ecological differences could also be present. We have segregated Type IV ponds based on their crustacean community composition, but this group could probably be split into two different habitat typologies if more samples were included. This type comprises sets of ponds in the bed of small intermittent streams, but also isolated ponds fed by water from springs in mountain slopes. Both kinds of ponds can dry up in some years, but in those associated with a stream bed, hydrochory may be an important source of recolonizing permanent waters species. Rareness curves and richness estimators confirm these assertions, indicating that species richness is underestimated for this type of mountain permanent ponds and also for temporary ponds in wet areas.

In conclusion, within a group of ponds under similar environmental constraints, species richness could be used to identify priority habitats for conservation. However, this parameter is not adequate when different habitat types are involved, unless a reference range of values is defined for each type.

### Conservation value

Species richness should not be the only criterion used for the assessment of conservation value because the characteristics of the species present are not considered (Fleishman *et al.*, 2006; Rosset *et al.*, 2012). Lower species richness, in a pond type, caused by adverse climate or any factors related to the intrinsic nature of the ecosystem, does not imply a degraded community. Temporary ponds hold a large number of exclusive species and taxa of high conservation value. This is not surprising because temporary habitats maintain specialized species that are able to cope with the restrictive environment. For example, the vast majority of large branchiopods are confined to these habitats and the isolation of these habitats can promote endemicity (Scheffer *et al.*, 2006). In the Iberian Peninsula, endemic branchiopods recorded by Alonso (1996) were found in singular aquatic habitats, such as saline and/or temporary waters. In the southern Iberian Mediterranean wetlands, rare crustacean species were mainly found in temporary water bodies (Gilbert e*t al.*, 2015). Thus, special ponds containing *Hemidiaptomus*, as well as genuine ephemeral ponds, stand out as hot spots for threatened relict or rare species.

The method proposed in this paper for scoring the conservation value of crustaceans is based on three criteria according to a hierarchical approach (Table 1). The first criterion takes into account the relictness and biogeographical significance of the major taxonomic group to which a species belongs. Large branchiopods and calanoids have higher scores; large branchiopods are primitive crustaceans characterized by morphological conservatism or bradytely and some of them have been used as flagship species of temporary ponds (Belk, 1998; Boven *et al.*, 2008). Despite this, their systematics remains incomplete (Brendonck *et al.*, 2008), although many endemic species have been described (Alonso & Jaume, 1991; Thiéry, 1988). In some countries (e.g., Austria; Eder & Hödl, 2002), selected locations are protected exclusively due to the presence of large branchiopods and some of its species have been included on the IUCN Red List, although Mediterranean species have not been yet incorporated. Among copepods, calanoids are remarkably well-adapted to drought and exhibit well-defined distribution patterns. Their worldwide distribution shows distinct clustering in major biogeographical regions (Dussart & Defaye, 2001, 2002; Hutchinson, 1967). Moreover, recent molecular studies suggest a high frequency of speciation events (Marrone *et al.*, 2010; Marrone *et al.*, 2013).

The second criterion ranks the species, (within the major groups) according to their confined distribution and/or endemicity to small areas. These two criteria want to account for the regional rarity, stressing the importance of "regional responsibility" in the conservation of species and sites (Schmeller *et al.*, 2008). Finally, the third criterion is based on the local rarity; the species are ranked according to the inverse of their occurrence in the study area. These criteria have been proven successful for prioritizing areas for the conservation of rare species (Gauthier *et al.*, 2010).

CCVI revealed the importance and singularity of temporary pond fauna by two estimators: the sum of species rarity scores and the averaged CCVI per species. When the evaluation is based on both species richness and conservation value, conservation values expressed per assemblage (i.e., the sum of all species scores) should not be used due to their redundancy with species richness (Rosset *et al.*, 2012).

CCVI is likely to penalize permanent ponds compared to temporary ponds, but the latter are more vulnerable and their loss is highly detrimental to the survival of relict species, which currently have a higher risk of extinction. However, the range of CCVI values used to define priority areas should be determined individually for each type of pond. The high scores attributed to large branchiopods, which are not present in permanent ponds, diminish the CCVI values when applied. CCVI values should be compared to a reference CCVI value specific to each pond type. The use of the ratio of the measured index to a reference-based predicted index is a common practice used to evaluate ecological status (Indermuehle *et al.* 2010). In a previous study we saw that the studied pond types contained nested communities (Sahuquillo & Miracle, 2013); therefore, the comparison of metrics for establishing priority habitats would be more meaningful when applied within pond types.

Thus, a generalized scoring method should be used as a complementary criterion to modulate species richness, in order to highlight the contribution of specialized or threatened species while diminishing that of opportunistic, widespread or invasive species.

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