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# Spatial distribution of *Branchinectella media* (Crustacea, Branchiopoda) in a saline pond from "La Mancha Húmeda": a case of habitat selection?

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## ABSTRACT

**Spatial distribution of *Branchinectella media* (Crustacea, Branchiopoda) in a saline pond from "La Mancha Húmeda": a case of habitat selection?**

*Branchinectella media* is a commonly observed anostracan in Mediterranean temporary saline ponds. Despite its wide distribution, many aspects of its natural history are unknown or poorly studied. In this work, we seek to understand the main factors that influence the spatial distribution of juveniles and adults of *B. media* in a pond that belongs to the Manjavacas Pond Systems in the "La Mancha Húmeda" Biosphere Reserve in central Spain. We sampled on two dates, 29 November and 24 December, 2015. Environmental variables, such as pH, conductivity, temperature, depth and vegetation cover, were recorded at 20 defined sites in the pond. A hierarchical partition analysis was used to identify the independent contribution of each variable on the density of juveniles and adults, and for each sampling date separately. On the first date, the predictive variables associated with the distribution of juveniles and adults were conductivity and depth, respectively. The density of juveniles was lower at sites with high conductivity, whereas adults occurred preferentially in deeper pond sectors. On the second date few adults were sampled, and only juvenile density was positively associated with depth. The use of deeper areas is possibly related to predation given the constant presence of waterfowl that feed on the littoral. Other causes, such as adaptation to temporary habitats with high evaporation rates and time stress to mate and produce resistant eggs, may play an important role.

**Key words**: Anostraca, environmental heterogeneity, hierarchical partitioning, temporary ponds

## RESUMO

**Distribuição espacial de Branchinectella media *(Crustacea, Branchiopoda) em uma lagoa salina de "La Mancha Húmeda": um caso de seleção de habitat?**

Branchinectella media *é um anostráceo comumente observado em lagoas salinas temporárias da região Mediterrânea. Embora amplamente distribuída, muitos aspectos da sua história natural são desconhecidos ou pobremente estudados. Neste trabalho, nós buscamos compreender os principais fatores que influenciam a distribuição espacial de juvenis e adultos de* B. media *em uma lagoa pertencente ao Complexo de Lagoas Manjavacas, Reserva da Biosfera "La Mancha Húmeda", centro da Espanha. Nós amostramos em duas datas, 29 de novembro e 24 de dezembro de 2015. Vinte pontos fixos foram definidos dentro da lagoa e, para cada ponto, as variáveis ambientais analisadas foram pH, condutividade, temperatura, altura da lâmina d'água e cobertura vegetal. Análise de Partição Hierárquica (HP) foi utilizada para identificar a contribuição independente de cada variável na densidade de juvenis e adultos em cada período de amostragem, separadamente. Na primeira data, as variáveis preditoras associadas significativamente a distribuição de juvenis e adultos foram condutividade e profundidade, respectivamente. A densidade de juvenis foi menor em pontos com maior condutividade enquanto que adultos ocorreram preferencialmente nos setores mais profundos da lagoa. Na segunda data o número de adultos amostrados foi baixo, e apenas a densidade juvenil foi positivamente associada à profundidade. O uso de áreas mais profundas está possivelmente relacionado com à predação, dada a presença constante de aves aquáticas alimentando-se no litoral. Outras causas, como uma adaptação a habitats temporários com altas taxas de evaporação e estresse temporal para*

*acasalar e produzir ovos resistentes podem estar desempenhando um papel importante.*

**Palavras chave**: *Anostraca, heterogeneidade ambiental, partição hierárquica, lagoas temporárias*

## INTRODUCTION

Habitat selection is the innate and learned process by which animals choose which habitat components (conditions, resources) to use (Hall *et al.*, 1997). It, therefore, results from the relationship between behaviour and environment, and it largely determines the local distribution of animal species (Meadows & Campbell, 1972). As it is almost universal, there are many studies, especially on birds, that have attempted to understand how animals continuously assess the suitability of their environment and move from place to place to take the best advantage of the range of conditions available to them (Cody, 1985). However, habitat selection differs from simple association as it implies choice, which is not always a straightforward assessment (Mayor *et al.*, 2009). In addition, habitat preferences may remain fixed throughout an animal's life span, or may alter depending on physiological state, age, origin, previous experience and learning, or past and present environments (Meadows & Campbell, 1972).

It is well-known that the distribution of aquatic organisms, such as zooplankters, is determined mainly by environmental heterogeneity (Pinel-Alloul, 1995) driven, in turn, by biotic or abiotic factors (Folt & Burns, 1999). Habitat patchiness can be associated with external factors, such as wind or water currents (Thackeray *et al.*, 2004). Contrarily, examples of active habitat selection include diel vertical and diel horizontal migration, which have been related mostly to predation avoidance (Timms & Moss, 1984; Neill, 1990; Lampert, 1993; Burks *et al.*, 2001; Castro *et al.*, 2007), but also to food availability, light or temperature preferences (Leibold, 1990; Leibold & West, 1993; Williamson *et al.*, 2011; Compte *et al.*, 2016). Fairy shrimps (Crustacea, Anostraca) are ancient and specialist inhabitants of temporary aquatic habitats that typically prevail under semiarid conditions (Brendonck *et al.*, 2008). They bridge dry periods or unfavourable conditions by producing resting eggs (Bren-

donck & De Meester, 2003; Brendonck *et al.*, 2016). They have a wide geographical distribution and occur in all continents, including the Antarctica (Weekers *et al.*, 2002; Toro *et al.*, 2006). The Iberian Peninsula currently houses 13 native species, plus one exotic introduction and two more species yet to be described (García de Lomas *et al.*, 2015). They inhabit almost the entire range of temporary aquatic habitats, from saline ecosystems to freshwater ponds (Cancela da Fonseca *et al.*, 2008; Gascón *et al.*, 2012; Machado & Sala, 2013). The species *Branchinectella media* (Schmankewitsch, 1873) belongs to the Chirocephalidae family and is frequently observed in saline temporary ponds (Alonso, 1996). Despite its wide occurrence, available information on its ecological preferences is scarce (Miracle, 1982; García *et al.*, 1997; Vekhoff, 1997; Alcorlo *et al.*, 2001; Mura *et al.*, 2005; Alonso, 2010; Ripoll-Rodriguez *et al.*, 2013; Marrone *et al.*, 2015).

Mediterranean temporary ponds are subjected to extreme and unstable ecological conditions, often isolated and which continually alternate between flooding and drying cycles, the duration of which can be highly variable (Grillas *et al.*, 2004; Olmo *et al.*, 2015; Brendonck *et al.*, 2016). Typically, the water phase starts with heavy rains filling ponds, which may involve one flooded phase, or several, during cooler seasons, and dry by water evaporation in summer. To overcome the dry phase, permanent residents depend on some type of drought-resistant life state (Williams, 2006). In addition, their life span does not necessarily correspond to the whole aquatic phase, and they can face time stress to mature and complete the life cycle before the habitat becomes unsuitable due to physical (drying, high salinity) or biotic (predation, competition, parasitism) factors (Wiggins *et al.*, 1980; Mura, 2003; Grillas *et al.*, 2004; Ripley *et al.*, 2004; Vanschoenwinkel *et al.*, 2010). The remarkable specialisation of highly complex biotopes placed Mediterranean temporary ponds under the protec-

tion of the Habitats Directive (92/43/EEC). Yet despite their high ecological relevance, Mediterranean temporary ponds are among the most threatened systems, with some endemic species disappearing at a high rate (Grillas *et al.*, 2004; Zacharias & Zamparas, 2010) due to drainage, river canalisation, water loss to agriculture, and especially wastewater from adjacent urban areas (Florin & Montes, 1999; Martinez-Santos *et al.*, 2008).

Habitat use patterns are not necessarily a reflection of choice, but can reveal resources and conditions that are important drivers of fitness, as well as distributions and population dynamics by extension. For this reason, examining habitat preferences is one way of assessing the importance of a habitat to species conservation. Here we investigate the habitat use patterns of *B. media* in a temporary pond in central Spain on two different dates and whether habitat preferences are similar between juvenile and adult states. We used a random sampling design to test if the spatial distribution differed among sites, and how it related to limnological conditions. With this study we aimed to advance in knowledge of large branchiopods in the Iberian Peninsula and to provide cues for conservation and management.

## MATERIAL AND METHODS

### Study site

The "La Mancha Húmeda" Biosphere Reserve in central Spain has numerous wetlands defined as "Important Bird Areas" due to the high occurrence of wintering and reproductive bird species. The region is also included as a Ramsar Site and is protected by Natura 2000. Alcahozo is a fishless temporary hypersaline pond located in this Reserve in the SE Iberian Peninsula (39°23'26.61"N, 2°52'37.37"W) in the province of Ciudad Real (Fig. 1). The climate there is Mediterranean with a continental character. Water inputs come from rain mainly, but also groundwater and runoff. The hydroperiod normally starts after strong autumn rains and ends in spring. The pond is located at 667.79 m a.s.l., and its maximum size is 88.6 ha and maximum depth is 0.84 m. The pond's vegetation consists of *Chara* spp, *Lamprotharmnium papulosum* and *Ruppia* spp (Bracamonte, 1982), and is basically distributed on the pond's western littoral. There is no record of macroinvertebrates in the bibliography, but large numbers of avian visitors are well-documented (Gosálvez *et al.*, 2012; Gonçalves *et al.*, 2016).
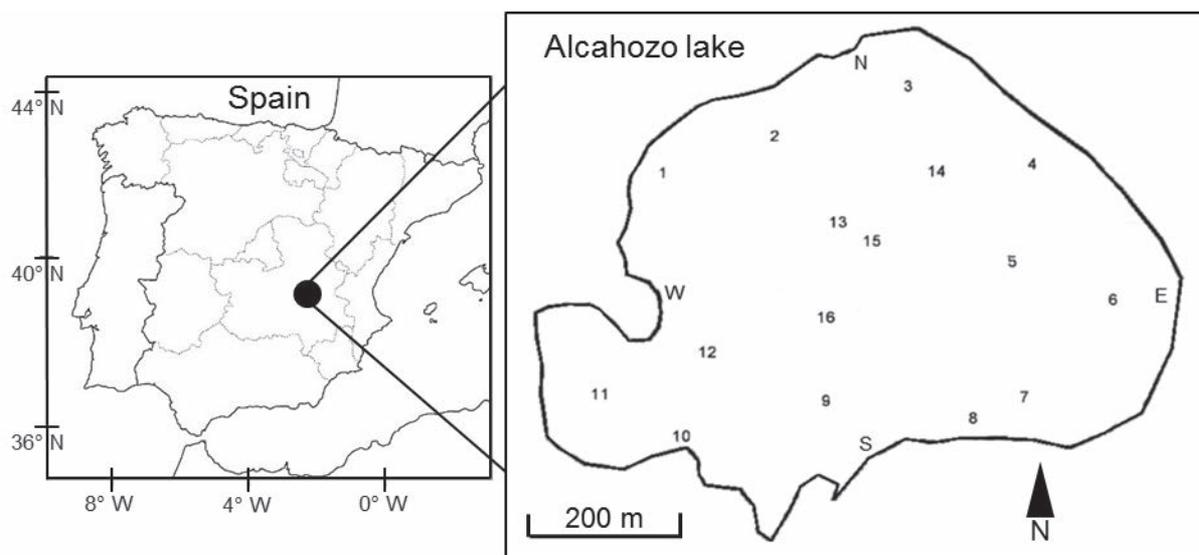


**Figure 1.** Map of the study area and distribution of the randomly chosen study sites in the Alcahozo pond. Numbers correspond to those in Table 1. *Mapa da localização da área de estudo e distribuição dos pontos aleatórios dentro da lagoa Alcahozo. Os números correspondem a Tabela 1.*

**Table 1.** Densities of the juveniles and adults of *B. media* and the limnological characteristics at the study sites on both sampling dates. Numbers correspond to those shown in Figure 1. Only the sampling sites with water at the time of the study are shown. CV (%) = Coefficient of variation. *Densidade de juvenis e adultos de* B. media *e as características limnológicas dos pontos estudados para as duas datas de amostragem. Números correspondem aos valores apresentados na Figura 1. Somente pontos com água são amostrados. CV (%) = Coeficiente de variação.*

| Dates Sites | Juveniles (ind/L) | Adults (ind/L) | Conductivity (mS/cm) | pH | Temperature (ºC) | Depth (cm) | Vegetation Cover (%) |
|---|---|---|---|---|---|---|---|
| 29/11/2015 | | | | | | | |
| 2 | 5.6 | 2.6 | 20.1 | 8.08 | 8.3 | 5.0 | 0.0 |
| 3 | 4.0 | 0.0 | 19.5 | 7.92 | 6.4 | 1.0 | 12.5 |
| 5 | 0.8 | 3.8 | 20.1 | 8.03 | 12.6 | 7.5 | 0.0 |
| 9 | 2.2 | 0.8 | 22.3 | 8.15 | 10.0 | 5.0 | 25.0 |
| 12 | 5.8 | 0.0 | 18.6 | 8.39 | 13.4 | 2.5 | 25.0 |
| 13 | 1.2 | 5.4 | 21.8 | 8.28 | 6.4 | 10.2 | 0.0 |
| 14 | 1.6 | 3.8 | 21.1 | 8.0 | 9.0 | 10.1 | 0.0 |
| 15 | 3.6 | 3.2 | 21.1 | 8.4 | 6.2 | 13.0 | 0.0 |
| 16 | 1.6 | 3.4 | 22.0 | 8.08 | 8.7 | 9.0 | 0.0 |
| W | 0.0 | 0.0 | 21.9 | 8.14 | 13.6 | 1.5 | 5.0 |
| E | 2.4 | 0.0 | 21.3 | 8.3 | 13.9 | 1.5 | 5.0 |
| N | 2.0 | 0.0 | 19.3 | 8.25 | 13.8 | 1.5 | 5.0 |
| S | 11.2 | 0.0 | 19.3 | 8.03 | 13.3 | 2.0 | 12.5 |
| Average | 3.2 | 1.8 | 20.7 | 8.2 | 10.4 | 5.4 | 6.9 |
| CV (%) | 91.8 | 111.3 | 6.0 | 1.9 | 29.7 | 77.1 | 132.8 |
| | | | | | | | |
| 24/12/2015 | | | | | | | |
| 2 | 0.6 | 0 | - | 8.57 | 14.3 | 2 | 25 |
| 5 | 11.6 | 0 | 34.4 | 8.46 | 13.6 | 5.5 | 12.5 |
| 9 | 0 | 0 | 34.5 | 8.48 | 13.6 | 2 | 0 |
| 13 | 1.8 | 1.4 | 33.9 | 8.61 | 11.3 | 8 | 0 |
| 14 | 6.6 | 0 | 32.8 | 8.48 | 12.8 | 6.5 | 0 |
| 15 | 5.4 | 0.8 | 33.3 | 8.56 | 10.5 | 10 | 0 |
| 16 | 5.4 | 0.2 | 35.3 | 8.52 | 11.2 | 5.5 | 0 |
| W | 1.2 | 0 | 35.3 | 8.6 | 14 | 2 | 5 |
| E | 1 | 0 | 34.2 | 8.3 | 13.8 | 2.2 | 0 |
| N | 0.4 | 0 | 33.5 | 8.51 | 13.4 | 2.5 | 0 |
| S | 0.2 | 0 | 33.7 | 8.51 | 13 | 3 | 5 |
| Average | 3.1 | 0.2 | 34.1 | 8.5 | 12.9 | 4.5 | 4.3 |
| CV (%) | 118.8 | 210.9 | 2.4 | 1 | 10.0 | 62.8 | 183.2 |

## Sample design and environmental variables

We randomised 16 sampling sites in the Alcahozo pond (Fig. 1). Firstly, the total pond area was divided into 16 blocks, with six squares each. We then randomly selected one square per block. The specific sampling points were allocated to the central region of each plot and covered a radius of an arm-length. Four more sites were defined at the four cardinal points on the border of the flooded area, defined as the maximum flooded area in normal years, identified by the limit of vegetation on the edge of the pond. Whereas random points were fixed on both dates, cardinal points were slightly adjusted during each sampling to include the pond boundary with water.

The hydroperiod at Alcahozo started in October 2015 and ended in May 2016. However, only two dates contained a sufficient sample size to perform a robust analysis of spatial distribution. The study took place on 29 November and 24 December, 2015. Sites were always sampled between 12:00 h and 16:00 h. At each site, conductivity, pH and temperature were measured (Hanna Instruments, Weilheim, Germany). In addition, the water depth and turbidity (when relevant) at each site were respectively determined with a scaled rod and a Secchi disc, and the percentage of vegetation cover was visually estimated at a 1 metre radius around the site point. We also visually checked for presence of birds or macroinvertebrates as indicators of potential predation.

At each site, we hauled 5 L of water with a plastic jar and filtered them through a 100 μm mesh size net. When the surface water was scarce, care was taken to neither include sediment nor to clog filters, and the presence and absence of anostracans were noted before sampling. The filtrate was immediately fixed with 70 % alcohol. Once in the laboratory, *B. media* individuals were taxonomically corroborated and identified as juveniles, males and females based on visible sexual characteristics (ovisac in females and penis in males) under a stereomicroscope at 4x.

## Statistical analysis

In order to avoid multicollinearity, we defined our environmental and population variables by excluding variables with Pearson's correlation r>0.7 (Mason & Perrault, 1991). As this criterion strongly correlates with depth, it allowed the removal of turbidity from the environmental variables. Similarly, the densities of males and females, which strongly correlated (r>0.7), were summed for each site and the "adults" variable was defined. The effect of the remaining five environmental variables was analysed for juveniles and adults densities separately. Correlation analyses were performed using the PAST software (Hammer *et al.*, 2001).

To determine the influence of the environment on the spatial distribution of *B. media*, we analysed the environmental variables associated with *B. media* density for the two sampling dates separately. For each period, we ran a hierarchical partitioning (HP) analysis (MacNally, 2000) and used $R^2$ as the goodness-of-fit measure to identify the variables that explained most of the *B. media* abundance across the different sites. In this analysis, all the possible models in a multiple regression setting that can be developed with a set of independent predictive variables and their explanatory power were segregated into the independent effect, I, along with the effects caused jointly with other variables, J. The independent contribution of each environmental variable and their significance were evaluated by tests based on 999 randomisations (MacNally, 2002). Linear regression models were used to determine the sign of each variable and the percentage of deviation from the model explanation (dispersion parameter for the Gaussian family). HP was conducted with the "hier.part" package in R (Walsh & MacNally, 2003) in v. 3.1.3 of the R-Program (R Development Core Team 2014).

## RESULTS

Not all the selected sites held water during the study: seven sites were dry on both sampling dates and two additional sites were dry on the second date. Among those sites with water, anostracans were found at them all, except for one on both dates (site W and site 9, respectively). We found juveniles and adults on both dates. Their averaged density (±standard error) over sites was 5 (±0.8) ind/L on the first date and 3.3 (±1) ind/L
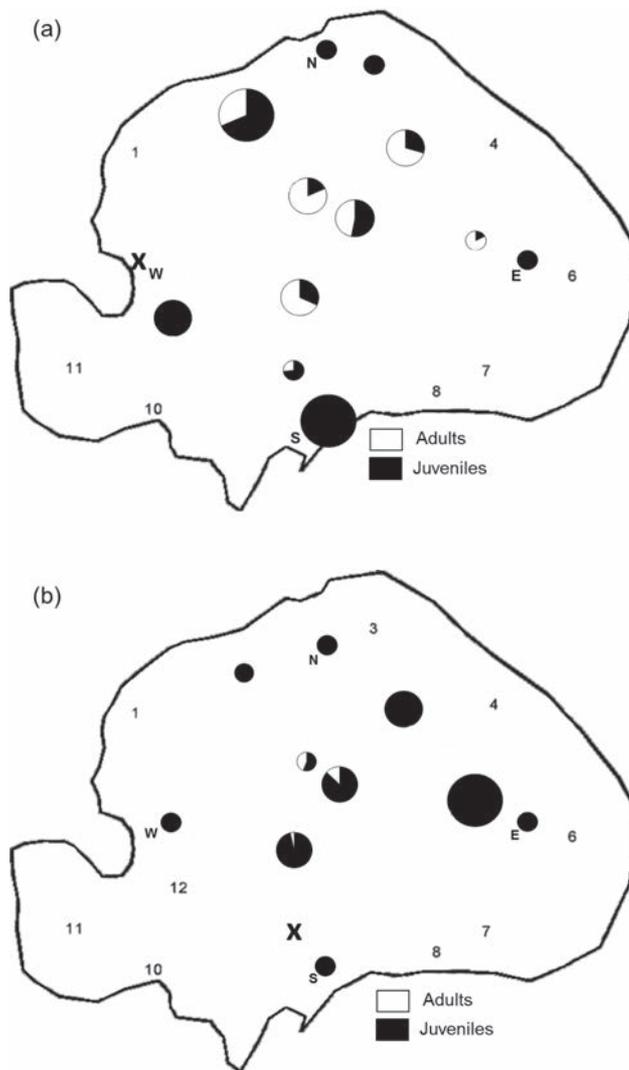
**Figure 2.** Distribution of *B. media* (adults and juveniles) at each site on the (a) first and (b) second sampling dates. Numbers and letters correspond to the sample sites in Figure 1 and Table 1. Samples found with no *B. media* individuals are numbered if dried, or marked with an "X" if they held water. Cardinal letters do not exactly coincide because their location was slightly adjusted to include water. The size of pie charts is proportional to the abundance in the 5-litre samples: small: <24 individuals, medium: 25-34 individuals, large: >35 individuals. *Distribuição de* B. media *(adultos e juvenis) em cada ponto da (a) primeira e (b) segunda coleta. Os números e as letras correspondem aos locais de amostra na Figura 1 e na Tabela 1. Pontos sem água apresentam somente números, ao passo que amostras com água, mas sem indivíduos de* B. media*, estão marcadas com um "X". As letras cardinais não coincidem exatamente porque sua localização foi ligeiramente ajustada para incluir água. O tamanho dos gráficos é proporcional à abundância encontrada em 5L: pequeno <24, médio: 25-34, grande >35.*

on the second. As shown in Figure 2, densities were normally lower on the littoral. We found adults mostly in the centre (deeper) part of the pond (Table 1, Fig. 2), whereas juveniles were found throughout the flooded area. Other zooplankters present in the samples besides *B. media* were the copepod *Arctodiaptomus salinus*

and the rotifer *Brachionus plicatilis*. We did not observe any macroinvertebrates on the study dates, but birds of the species *Calidris alpina, C. minuta*, and *Charadrius alexandrinus* were commonly seen to be feeding on the pond's littoral.

Table 1 shows the limnological characteristics recorded on both dates. Interestingly, all the

**Table 2.**  Results of the hierarchical partitioning analysis showing the independent contribution of each variable on density (ind/L) of the *B. media* juveniles and adults on two sampling dates. *% Dev* is the percentage deviation obtained from a linear regression model. *I* is the independent contribution and *J* the joint contribution of each variable. *% I* is the percentage contribution of one predictor variable explained by a model including all the variables jointly. The sign of each variable was assessed from linear regression models. *Z-score* refers to the randomisation test calculated from 999 randomisations for the independent contributions of each variable. *\*p<0.05. Resultados da análise de Partição Hierárquica mostrando as diferentes contribuições de cada variável na densidade (ind/L) de juvenis e adultos de* B. media *nas duas datas de coleta.* % Dev *corresponde à porcentagem de desvio obtido a partir do modelo de regressão linear.* I *corresponde à variação independente e* J *à contribuição conjunta de cada variável.* % I *corresponde à porcentagem de contribuição de uma variável preditora no modelo, incluindo todas as variáveis juntas. O sinal de cada variável foi obtido a partir de regressão linear.* Z-score *se refere ao teste com 999 randomizações a partir das contribuições independentes de cada variável.*

| Date / Variables | Juveniles | | | | | Adults | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *%Dev/Sign* | *I* | *J* | *%I* | *z-score* | *%Dev/Sign* | *I* | *J* | *%I* | *z-score* |
| **29 Nov 2015** | 43% | | | | | 86% | | | | |
| Conductivity (mS/cm) | - | 0.30 | 0.06 | 70.5 | 2.19* | | 0.05 | 0.11 | 6.19 | -0.31 |
| pH | | 0.006 | -0.0001 | 1.38 | -0.82 | | 0.01 | -0.009 | 1.18 | -0.61 |
| Temperature (ºC) | | 0.008 | 0.003 | 1.88 | -0.82 | | 0.14 | 0.22 | 16.4 | 0.54 |
| Depth (cm) | | 0.03 | 0.05 | 7.45 | -0.60 | + | 0.47 | 0.32 | 55.1 | 5.68* |
| Vegetation cover (%) | | 0.08 | 0.05 | 18.7 | -0.11 | | 0.18 | 0.22 | 21 | 1.19 |
| **24 Dec 2015** | 74% | | | | | 64% | | | | |
| Conductivity (mS/cm) | | 0.06 | -0.06 | 8.87 | -0.23 | | 0.01 | 0.01 | 2.14 | -0.84 |
| pH | | 0.02 | -0.02 | 3.38 | -0.66 | | 0.14 | 0.17 | 21.9 | 0.26 |
| Temperature (ºC) | | 0.15 | -0.10 | 20.3 | 0.55 | | 0.24 | 0.32 | 37.1 | 1.13 |
| Depth (cm) | + | 0.37 | -0.08 | 50.4 | 2.31* | | 0.22 | 0.31 | 34.2 | 1.06 |
| Vegetation cover (%) | | 0.12 | 0.10 | 16.8 | 0.02 | | 0.02 | 0.06 | 4.47 | -0.58 |

physical water characteristics presented a wider rank among those sites from the first sample and were more homogeneous on the second. Temperature differed by 7.7 ºC among sites on the first date compared to 3.8 ºC on the second. All the ranks overlapped at some point between dates, except for conductivity with a maximum of 22.02 mS/cm on the first date and a minimum of 32.80 mS/cm on the second.

The HP results are shown in Table 2. Juveniles showed different responses to the set of variables explored during both sampling periods, while adult distribution was only significant on the first date. Of the five variables included in the analysis, conductivity better explained the juvenile distribution on the first date (Table 2; Fig. 3), with an independent contribution of 70.5 %, and depth was the most important variable for the distribution of adults in the first sample (55.1 %) (Table 2; Fig. 3), and of juveniles in the second (50.4 % of independent contribution) (Table 2; Fig. 4).

## DISCUSSION

The spatial distribution of *B. media* at the Alcahozo pond was determined mainly by depth and
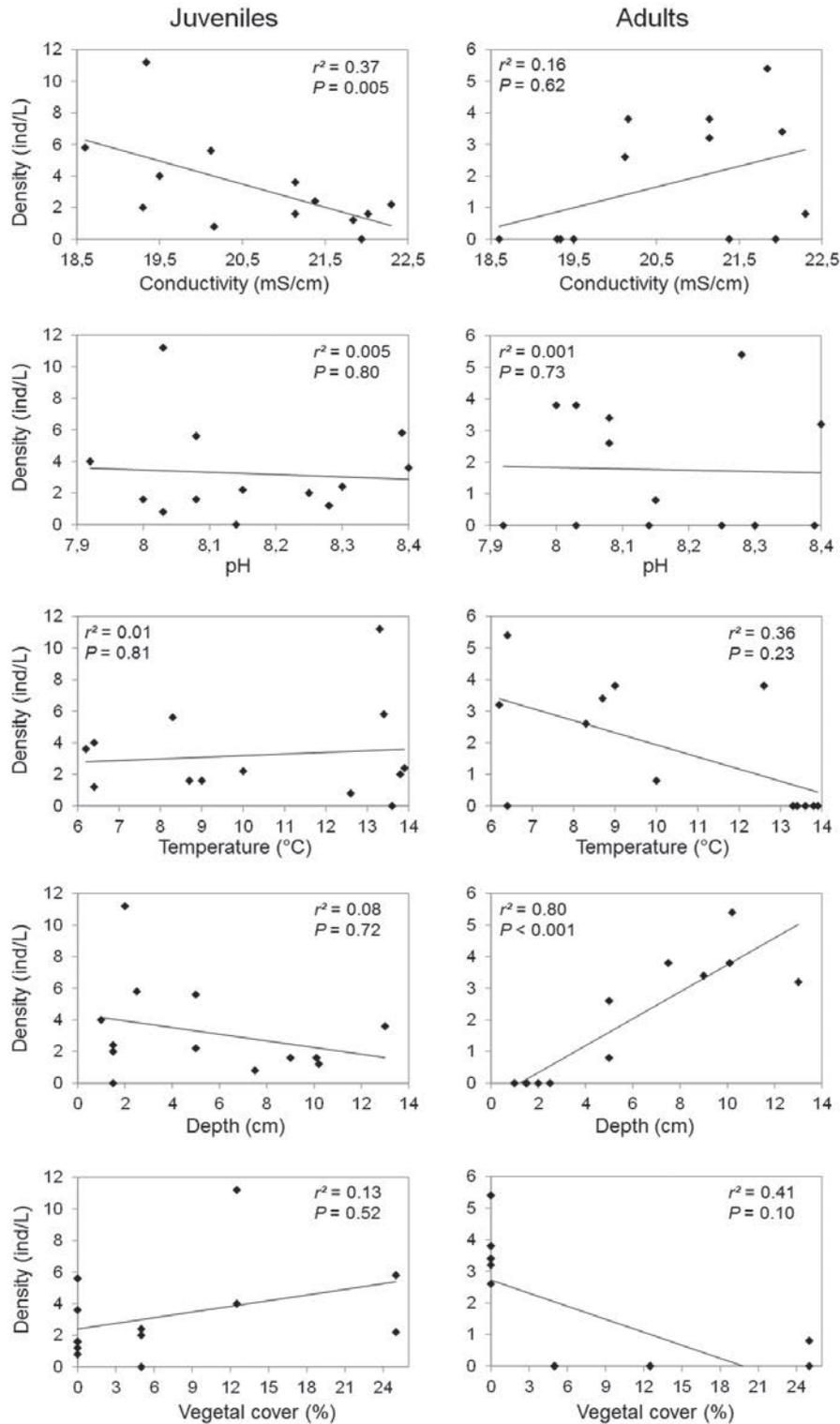
**Figure 3.**   Relation between *B. media* density (ind/L) and the studied environmental variables on 29 November 2015. *Relação entre a densidade de* B. media *(ind/L) e as variáveis ambientais estudadas em 29 de Novembro de 2015.*

conductivity. The relationship of the predictor variables with the density of juveniles and adults on both sampling dates differed. During the peak abundance, distribution of juveniles was influenced by conductivity, whereas that of adults was influenced by depth. At the start of population decline, depth was relevant only for the juvenile stage, but we ought to take into account that average depth was lower and more sites had dried at that time. Moreover, the lack of influence of depth on adults was probably the result of the excessively small sample size and the consequent low resolution power. On both dates, adult individuals mostly used the centre of the pond, which was also the deepest area. We believe that this behaviour is strongly related to predation pressure. Hundreds of birds fed on the pond's shore during the hydroperiod (Gonçalves *et al.*, 2016). These were waders of no more than 20 cm, and the centre of the pond was too deep for them to feed. As we did not observe macroinvertebrates, since they are rare in this type of saline systems (Waterkeyn *et al.*, 2008), birds seemed to be their main predators. In fact anostracans have been reported as keystone feeding species for the spring and autumn migrations of waterfowl (Horváth *et al.*, 2013b), and their importance as a unique food resource in saline ecosystems is widely known (Roberts, 2013). Predation is known as an important biotic stress that regulates temporary pool communities (Brendonck *et al.*, 2002), and anostracans' habitat has been frequently described as restricted due to their high sensitivity to predation (Alonso, 2010). It has been suggested that crustacean zooplankton displays flexible, predator-sensitive migration patterns (Neill, 1990). Predation avoidance has been related to diel vertical migration (Neill, 1990; Lampert, 1993; Burks *et al.*, 2001; Castro *et al.*, 2007), but depth at Alcahozo is too limited. Horizontal migration is also a recurrent strategy, especially if macrophytes serve as potential refuges for fish predation (Schriver *et al.*, 1995; Lauridsen & Buenk, 1996; Burks *et al.*, 2001). However, macrophytes may not provide adequate refuge to zooplankton in shallow Mediterranean fishless ponds (Castro *et al.*, 2007; Compte *et al.*, 2016), and the vegetation cover in our pond was scarce and air-exposed. Finally, some studies have

included avoidance of shores as a result of predator-induced aggregations (Romare & Hansson, 2003; Pinel-Alloul *et al.*, 2004). In our study it is not clear whether adults actively avoided the shore or if their absence on the littoral was the result of selective predation on adults. The latter hypothesis may be solved by investigating the preferred prey size of the bird species that normally visit the pond. Further evidence suggestive of the former hypothesis (habitat selection) comes from the fact that most juveniles on the second date were also found in deeper areas. Besides predation avoidance, migrating beyond a shallow area to a deeper part of the pond could be a response to environmental cues by anticipating the onset of adverse conditions (high UV, high salinity, high temperature, imminent drought). This would imply an adaptation to temporary ponds that frequently dry out through water evaporation, which is the typical habitat of anostracans. Moving to a higher water volume may not only imply a better chance of survival, but also more opportunities of finding a mate, and more possibilities of completing the life cycle and producing resting eggs to survive the dry period (Vanschoenwinkel *et al.*, 2010). We cannot completely rule out a third possibility: adults actively avoided being captured on the littoral as shallower waters obliged a slower iterative hauling process. However, water transparency allowed us to confirm the absence of adults before sampling.

Although this study was purely spatial, sampling in two different months at the start of the growing season allowed us to confirm that the system was highly dynamic. We observed how size generally reduced with time, but we also confirmed that this reduction was not homogeneous as some areas exposed on the first date were submerged on the second (P. Pons, *pers. obs.*). This could explain the presence of juveniles in this population 1 month after their first hatch.

Reduced depth and pond size (seven sites versus one had dried on the second date) explained the higher conductivity values recorded for the second samples due to higher evaporation. Conductivity as a proxy for salinity is a crucial factor for determining species composition (Boix *et al.*, 2005; Angeler *et al.*, 2008; Nhiwatiwa *et al.*, 2011; Horváth *et al.*, 2013a). In Mediterranean
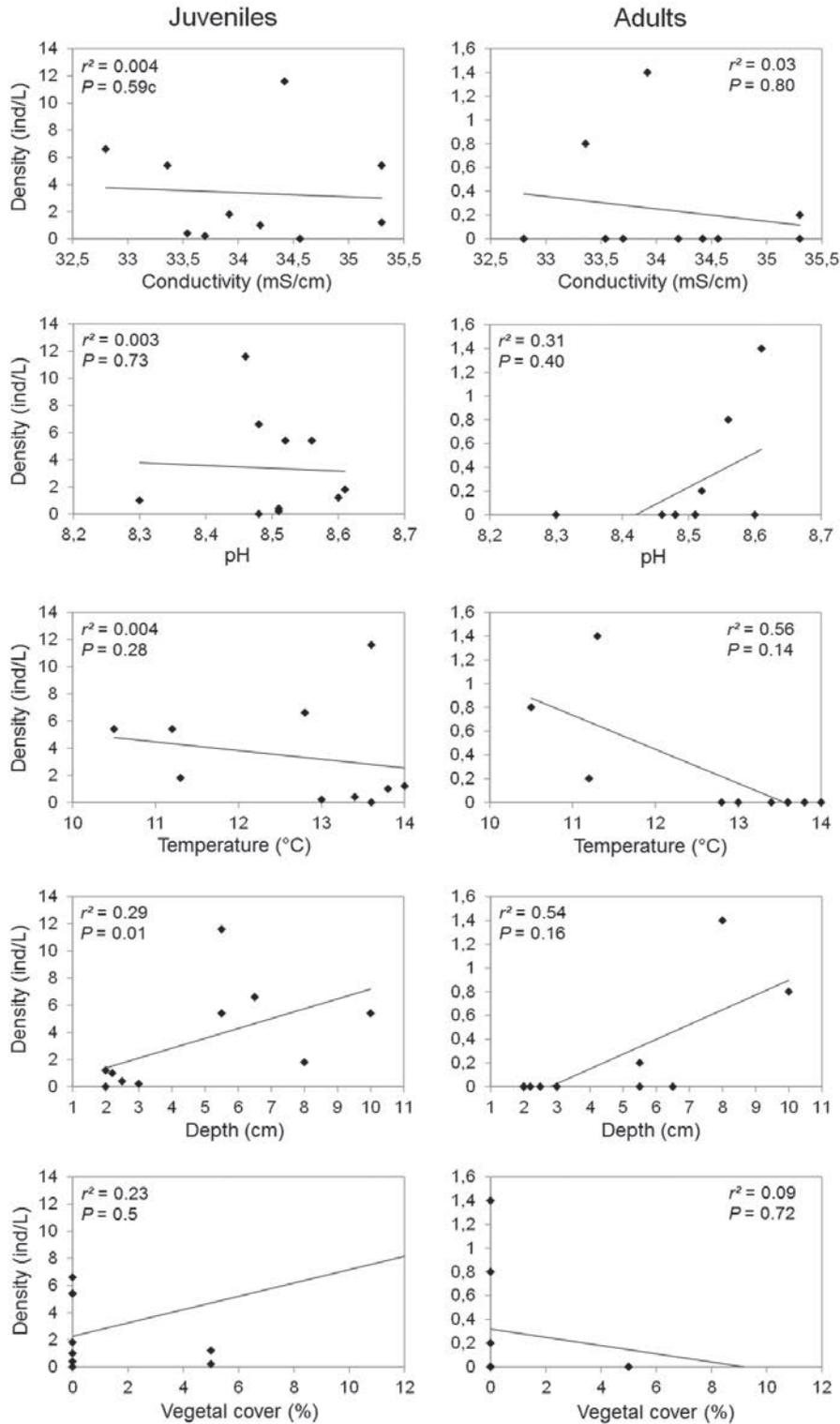
**Figure 3.** Relation between *B. media* density (ind/L) and the studied environmental variables on 24 December 2015. *Relação ente a densidade de* B. media *(ind/L) e as variáveis ambientais estudadas em 24 de Novembro de 2015.*

ponds, decreased species richness has been found with increasing salinity (Boronat *et al.*, 2001; Green *et al.*, 2005; Brucet *et al.*, 2009; Boix *et al.*, 2008; Waterkeyn *et al.*, 2008, 2009). In line with these studies, our study revealed a constrained community of little complexity: three species typical of saline environments. These species are probably locally adapted to the pond's naturally high salinities but, as suggested by Horváth et al. (2013a), they might be more tolerant than specialised to these highly saline waters as they also occur at the lower end of the gradient. In fact *B. media* hatches more at lower conductivities (Pons, *pers. obs.*). We detected conductivity as an important abiotic factor which influenced the spatial distribution of *B. media*, but it was significant only for juveniles on the first sampling date. The reason for this may rely on a differential response between the two life stages. In fact some authors have suggested greater sensitivities to salinity for the first anostracan life cycle stages (Waterkeyn *et al.*, 2009). Another possibility may be associated with the among-sites salinity differences between dates as conductivity on the first date varied from 18.6 to 22.3 mS/cm, whereas a general high salinity condition over the pond was observed on the second date (32.8 to 35.30 mS/cm). As a result, juveniles were more abundant at low salinity sites on the first date, but showed no preferences on the second. Maybe conductivity values were so high on the second date that the physiological benefits from occupying sites with lower values were not significant. An experimental approach to the sensitivity differences between *B. media* juveniles and adults would be extremely valuable.

Here we demonstrate that the anostracan *B. media* is spatially distributed according to depth and conductivity patterns in two snapshot samples as a first step to elucidate the ecological requirements of this species. Whether this distribution responds to habitat selection or to a simple association requires further exploration. Future studies on a temporal survey across the hydroperiod and experiments to specify their ecological preferences across the life cycle are clearly needed to better understand the biology of this keystone species of saline environments.

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