KUNGL.
VETENSKAPS-
AKADEMIEN
THE ROYAL SWEDISH ACADEMY OF SCIENCES

CrossMark

REPORT

# Human-mediated dispersal of aquatic invertebrates with waterproof footwear

Luis Valls, Andreu Castillo-Escrivà, Francesc Mesquita-Joanes,
Xavier Armengol

**Abstract** Human-mediated dispersal has rarely been considered in wetland conservation strategies at regional scales, yet high concern exists about this aspect for (inter-)national management considering invasive species in other aquatic systems. In this context, we aim at understanding the role of human-mediated dispersal by footwear in protected wetlands with high conservation value. Zooplankton and zoobenthos were sampled in 13 shallow lakes in central Spain and, at the same time, mud attached to waders was collected and later cultured in deionized water under laboratory conditions for 4 weeks. Two-hundred and four individuals belonging to 19 invertebrate taxa were recovered after hatching; Ostracoda (84 %), Cladocera (53 %), Copepoda (30 %), Anostraca (30 %), and Notostraca (7 %) were the most frequent groups among the hatched crustaceans. NMDS and PERMANOVA analyses showed significant differences between the dispersed (via footwear) and the source active metacommunity, suggesting different dispersal abilities among the species found. Human vectors facilitate dispersal among protected lakes, which could eventuality lead to biotic homogenization and faster spread of non-indigenous species. Preservation strategies and education campaigns associated to target humans in close contact with water bodies should be imperative in conservation management of protected lakes.

**Keywords** Conservation management ·
Human-mediated dispersal · Invasive species ·
Protected lakes

## INTRODUCTION

Historically, human populations have been crossing seas and oceans for millennia (e.g., Strasser 1999; Derricourt 2005). Besides domesticated animals and plants, other organisms associated with human watercraft, boots, and clothes can be unintentionally transported making the first potential events of long-distance dispersal by non-natural mechanisms (Carlton 1999). Over the past two centuries, transport movements increased exponentially, being more international and faster than ever. These human-mediated dispersal events have resulted in a global problem of alien species invasions of great international concern (Auffret et al. 2014).

Thousands of tourists but also many scientists come from near and far to visit protected areas, a human activity commonly permitted even encouraged in conservation management (Worboys et al. 2005). In these cases, visitors could act as unintentional vectors introducing organisms in the protected area or dispersing local species elsewhere (Pickering and Mount 2010). In addition, they may represent a new dispersal mechanism in the metacommunity dynamics for patched zones or for a set of isolated habitats in the same protected area (Auffret et al. 2014). In both cases, human-mediated dispersal can represent a significant problem to the preservation of the pristine conditions in areas with high conservation value (Pickering et al. 2011). Examples of documented invasions in inland waters include the case of North American Great Lakes (Mills et al. 1994) but also others by crustaceans such as *Daphnia lumholtzi* G.O. Sars 1885 (Havel et al. 1995), *Daphnia pulex* Leydig, 1860 (Mergeay et al. 2005), and *Artemia franciscana* (Kellog 1906) (Amat et al. 2005).

In the last decades, research on the role of humans as dispersers has notably risen (Auffret et al. 2014). Alien

Springer

plants and invertebrates are among the main target groups of research on human-mediated dispersal (e.g., Griffiths et al. 1991; Ansong and Pickering 2014). The majority of these studies focused on commercial transport (of goods such as seeds, soil, aquaria, and machinery) and on organisms attached to boats or in ship ballast waters (e.g., Carlton 1999; Reichard and White 2001; Escrivà et al. 2012). This is mainly due to the rise of dispersal events with the increase of international commerce, now further expanded through the use of internet (Lenda et al. 2014); e-commerce delivers, over new and traditionally routes, different species to far away places producing an increase of long-distance dispersal events. However, few studies focused specifically on the impact of clothes and footwear usage as unintentional dispersal vectors. A representative example is the study by Pickering et al. (2011), who investigated the impact of tourism as a dispersal vector of plants in a protected area of Australia. Similar experiments support the importance of clothes and car wheels in seed dispersal (Pickering and Mount 2010). But analogous studies involving other groups, such as aquatic invertebrates, are still scarce.

Aquatic invertebrates, and crustaceans in particular, are represented by a high number of species in shallow lakes. Many zooplanktonic and zoobenthic species produce dormant eggs to survive periods of unfavorable conditions (Alekseev et al. 2007). These eggs accumulate at the bottom of lakes, building the so-called dormant egg bank (Hairston et al. 1995). Nevertheless, only the first few centimeters of the sediment, where eggs may hatch with stimuli (salinity, temperature, light, etc.) constitute the active egg bank (Cáceres and Hairston Jr. 1998). Human activities around water bodies (tourism, research, and recreational activities) interact with this sediment (Fig. 1). Dormant eggs could therefore be dispersed by humans, which can then affect the dispersal dynamics of freshwater invertebrates (Muñoz 2010; Auffret et al. 2014). Although there are many reports of human footwear as dispersers of seeds in plants (Pickering and Mount 2010), only a few studies have dealt with aquatic invertebrates being dispersed by humans walking on egg-filled lacustrine sediments.

A recent approach to the study of human-mediated dispersal by waterproof boots estimated, for the first time, the potential of this mechanism to disperse aquatic invertebrates and the implications for aquatic metacommunities (Waterkeyn et al. 2010a). Anostraca, Ostracoda, and Cladocera were the groups that hatched in higher proportions of individuals, but the Protozoa (88 %) and Ostracoda (40 %) were the most prevalent in the analyzed samples. Waterkeyn et al. (2010a) found all of these groups to be present in the egg bank of water bodies in the study area,

and demonstrated that invertebrate propagules were transported by the sediment attached to footwear. This nonstandard vector may be an important mechanism connecting biogeographic islands and affecting metacommunity dynamics.

Besides the potential dispersal offered by footwear, this might be notably enhanced by a superimposed transport vector to cover larger distances (car, plane, ship, etc.). This entails the possibility of long-distance dispersal (Waterkeyn et al. 2010a) and a potential invasion of alien species in a new territory. In this way, a recent study demonstrated how scientific activities can be a successful source for the dispersal of alien species by footwear; *Candona furtosae* Teeter 1980, a species only distributed in Florida, hatched in Spain from the sediment accumulated in boots that had been used by participants in an International Biogeography Society meeting (Valls et al. 2014a).

Human-mediated dispersal is often overlooked in management strategies of continental water bodies. The studies above and the high presence of human activities in aquatic environments highlight human-mediated dispersal by footwear as a mechanism to be taken into consideration in invasion biology and conservation management. Understanding the role of this vector in aquatic communities is essential for their preservation (Muñoz 2010). In this framework, we provide a quantification of human-mediated dispersal by footwear in a protected wetland area. We focused our work on the potential dispersal of aquatic invertebrates (mainly on microcrustaceans) via human visitors, in order to understand their impact on the alteration of dispersal routes in lake metacommunities.

## MATERIALS AND METHODS

### Study site

The shallow saline lakes of the Biosphere Reserve of "La Mancha Húmeda," located on the southern sub-plateau of the Iberian Peninsula, are unique ecosystems in the Palearctic (Armengol et al. 1975; Florín and Montes 1999). It is the largest steppic wetland area of Western Europe, encompassing 30 000 ha and including 12 protected areas (e.g., Tablas de Daimiel and Ruidera lakes). Seasonal, intermittent, and semi-permanent lakes with a rich biodiversity and fragile conservation status are the focus of conservation management. Nevertheless, some of them have a hydrological regime disturbed by human activities. Urban waste water spilled into the lakes from near localities and the reduction of groundwater level through aquifers overexploitation by agriculture are leading problems for their preservation (Florín and Montes 1999).

**Fig. 1** Picture taken at a saline lake (Peñahueca) of "La Mancha Húmeda" Biosphere Reserve illustrating foot tracks done during sampling

## Sampling, experimental design, and sample processing

In December 2012, two kinds of samples were surveyed in 13 lakes (Fig. 2), some of them with a temporary hydrological regime. Zooplankton and zoobenthos samples were collected from the different microhabitats at the center of the lake (reached by foot with waders) using hand nets. For zooplankton, we used a 20-cm-diameter and 65-μm mesh size net, filtering a total volume of approximately 0.6 m³. Zoobenthos was collected using a rectangular (25 × 20 cm) hand net, with a 250-μm mesh, swept over all different substrates observed (up to an approximate substrate area of 2 m²). Samples were immediately fixed with 4 % formaldehyde.

In order to perform a hatching experiment, after sampling zooplankton and getting out of the lake (Fig. 1), mud samples were collected with spatulas from the soles of waders used by one person (#43, European size). Each sample was stored separately in a plastic bag. Then, the rest of the mud attached to the waterproof boots was cleaned with spatulas and distilled water to reduce the risk of contamination to the next lake. After being dried at room temperature and stored for a 6-month resting period in dry, cool (4 °C), and dark conditions, sediment from each sample was placed in a Petri dish inside an aquarium filled with 1 L of deionized water. The experiment ran for 24 days. The amount of sediment obtained was not the same for all samples; when the (wet) sediment obtained for each pair of boots weighed more than 50 g, 3 replicated aquaria were set. In these cases, the sediment was fractioned in 3 proportional parts. For the remaining samples, we prepared only one aquarium with all of the mud obtained from the boots. As a control treatment, we cultured 3 aquaria without sediment. Aquaria were placed in a culture chamber at a constant temperature of 20°C and 10 h light: 14 h dark photoperiod. Periodically, the content of each aquarium was filtered through nylon nets with mesh size of 100 μm and the observed living organisms were extracted and counted under a stereomicroscope. All the aquaria were sampled 6 times over 4 weeks (days 1, 4, 8, 12, 16, and 23 since the onset of the experiment). Juveniles captured that could not be identified (mostly Ostracoda) were cultured in separate 100-mL microaquaria at the same culturing conditions and grew up to obtain adults for a correct identification (unless they died prior to attaining adulthood). These individuals were fed on mixed food following Schmit et al. (2007). Conductivity, oxygen content (at day and night), and pH were measured on three occasions (days 2, 13, 24) in each aquaria. Specimens collected were identified to species level according to Dussart (1967), Alonso (1996), and Meisch (2000).
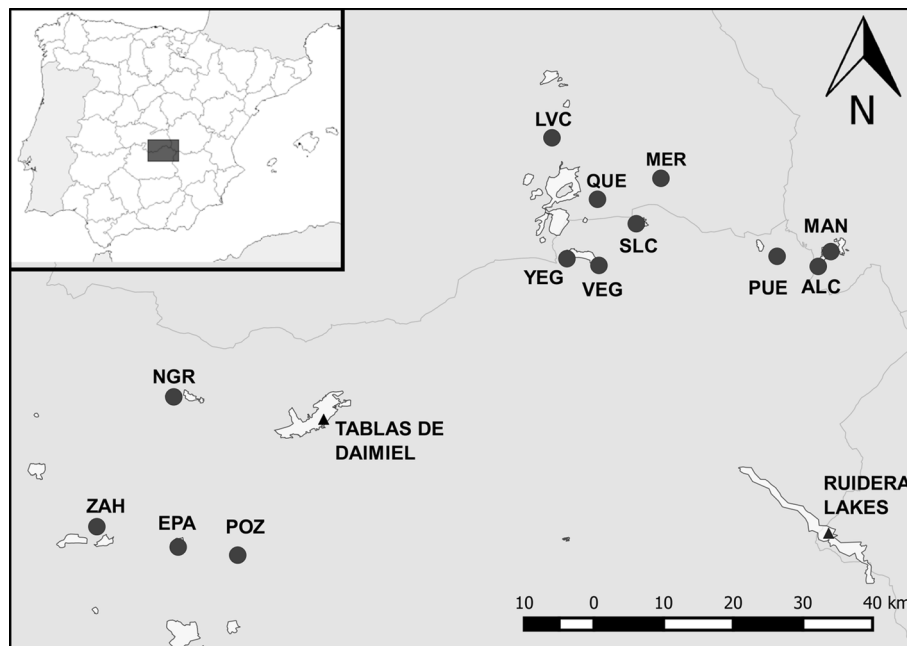


**Fig. 2** Map of the study region and geographical locations of lakes sampled (*dots*). *Light gray zones* show water bodies included in "La Mancha Húmeda" Biosphere Reserve. Two close areas with high conservation value are also indicated: Tablas de Daimiel National Park and Ruidera lakes Natural Park

## Statistical analyses

Multivariate statistics were used to compare the crustacean community composition obtained in the field with the composition resulting from hatching experiments. Two non-metric multidimensional scaling (NMDS) analyses were carried out to assess the main patterns in the invertebrate assemblages: one using the data matrix at the species level, but excluding information on unidentified juveniles from the dataset, and other including data on these juveniles but pooling together taxa at the family level or above to be able to compare the whole dataset although at a lower taxonomic resolution. In NMDS, Hellinger distances were calculated from the biological matrices based on presence–absence data.

Royston's multivariate normality tests were used to check the biological matrix, which showed a non-normal distribution. Consequently, we ran the 'adonis' function in R (see below) to do a permutational multivariate analysis of variance (PERMANOVA) which was carried out on Hellinger distances applied to presence–absence data from the biological matrix (including juveniles and at family level or above) to compare the taxonomic composition between the metacommunity hatched from boots mud (e.g., the set of dispersed communities) and that sampled directly from the lakes (the set of active communities). In addition, for both datasets, i.e., using or not juveniles, two similarity percentage analyses (SIMPER) were carried out to examine which species contributed most to the differences between dispersed and source active metacommunities. To check for differences in oxygen content in the experiment between day and night, a pairwise t test was used. We also calculated the Spearman correlation between the lake environmental parameters (conductivity, oxygen content, and pH) and the

Hellinger distance calculated between dispersed and their respective source active communities.

In the hatching experiment, we estimated the index for timing of hatching (ITH), calculated as the median number of days (incubation period) needed by a specific taxon to hatch (Vandekerkhove et al. 2004) and the median velocity of hatching (MVH), as the median number of individuals hatched in each aquarium divided by the total experimental time (number of days).

All statistical tests and ordination analyses were performed in R ver. 2.12 (R Development Core Team 2013) using libraries vegan (Oksanen et al. 2015), stats (R Development Core Team 2013), MVN (Korkmaz et al. 2014), and labdsv (Roberts 2013).

## RESULTS

No individuals hatched in the control samples. Electrical conductivity (Table 1) increased throughout the experiment and aquaria ended with a wide range of values (0.26–4.86 mS cm$^{-1}$). No significant differences were determined in the oxygen measures between day and night ($p > 0.05$). A total of 204 individuals and 19 invertebrate taxa hatched during the experiment (Table 2). Invertebrate hatchlings included mainly Ostracoda (84 %), Cladocera (53 %), Copepoda (30 %), Anostraca (30 %), and Notostraca (7 %). The species *Dunhevedia crassa* King 1853, *Alona salina* Alonso 1996, and *Leydigia acanthocercoides* (S. Fischer 1854) were collected from the experimental aquaria but not directly from the lakes (Supplementary material S1). Hence, 15 % of the hatched taxa were found only in the experimental setting. On the other hand, 34 invertebrate species were detected in the lake active communities, but in

**Table 1** Physicochemical and hatching parameters measured from the experimental aquaria. *Density* individuals hatched per gram of wet sediment attached to waders, *MVH* median velocity of hatching, *Lake cond.* conductivity in the lake, *Cond.* electrical conductivity in the aquaria

| Lake | Codes | Density (Ind. g$^{-1}$) | MVH (Ind. day$^{-1}$) | Cond. (mS cm$^{-1}$) | Lake cond. (mS cm$^{-1}$) | pH | O$_2$ range (% Sat.) |
|---|---|---|---|---|---|---|---|
| Pueblo | PED | 2.39 | 6.33 | 0.3 ± 0.15 | 5.2 | 7.6 ± 0.9 | 55–83 |
| Alcahozo | ALC | 1.04 | 2.16 | 1.3 ± 0.78 | 21.7 | 7.5 ± 0.9 | 63–77 |
| La Veguilla | LVE | 0.14 | 2.33 | 0.3 ± 0.07 | 2.6 | 7.7 ± 1.0 | 60–85 |
| Mermejuela | MER | 0.19 | 0.33 | 1.5 ± 0.58 | 115.1 | 7.3 ± 0.9 | 66–90 |
| Nava Grande | NGR | 0.04 | 0.33 | 0.4 ± 0.19 | 2.2 | 7.6 ± 0.8 | 65–88 |
| El Pardillo | EPA | 0.05 | 1 | 0.1 ± 0.06 | 0.0 | 8.0 ± 0.6 | 69–80 |
| Larga Villacañas | LAV | 0.22 | 0.16 | 1.2 ± 0.68 | 23.5 | 7.6 ± 0.7 | 68–81 |
| Manjavacas | MAN | 1.07 | 5.5 | 0.3 ± 0.17 | 9.7 | 7.8 ± 0.8 | 67–135 |
| Pozuelo | POZ | 0.12 | 0.66 | 1.1 ± 0.67 | 11.9 | 7.6 ± 0.8 | 63–83 |
| Quero | QUE | 0.02 | 0.33 | 3.1 ± 1.58 | 87.5 | 7.5 ± 0.8 | 42–80 |
| Salicor | SLC | 3.32 | 19 | 1.7 ± 1.06 | 62.8 | 7.4 ± 0.6 | 57–80 |
| Yeguas | YEG | 0.00 | 0 | 3.6 ± 1.66 | 106.2 | 7.8 ± 0.4 | 23–80 |
| Zahurdones | ZAH | 0.16 | 4 | 0.2 ± 0.12 | 0.4 | 8.3 ± 0.2 | 63–80 |

**Table 2** Number of individuals and prevalence (percentage of samples) for species and higher level taxonomic groups hatched from mud samples collected from boots. Symbol + indicates that taxa could not be reliably quantified. *ITH* index for timing of hatching (after Vandekerkhove et al. 2004)

| Taxa | | Number of hatchlings | Prevalence (%) | ITH (days) |
|---|---|---|---|---|
| Anostraca | | | | |
| *Artemia parthenogenetica* | | 2 | 8.3 | 8 |
| Anostraca sp. juveniles | | 21 | 25 | 11 |
| Notostraca | | | | |
| *Triops cancriformis* | | 4 | 8.3 | 13 |
| Cladocera | | | | |
| *Daphnia mediterranea* | | 1 | 8.3 | 23 |
| *Daphnia magna* | | 3 | 16.6 | 19 |
| *Daphnia* sp. juveniles | | 6 | 16.6 | 18 |
| *Leydigia acanthocercoides* | | 1 | 8.3 | 18 |
| *Alona salina* | | 6 | 16.6 | 18 |
| *Dunhevedia crassa* | | 3 | 16.6 | 18 |
| *Pleuroxus letourneuxi* | | 12 | 25 | 22 |
| Eurycercidae sp. juveniles | | 7 | 33.3 | 21 |
| Ostracoda | | | | |
| *Eucypris virens* | | 9 | 25 | 21 |
| *Heterocypris barbara* | | 9 | 8.3 | 23 |
| *Heterocypris incongruens* | | 2 | 8.3 | 18 |
| *Sarscypridopsis aculeata* | | 1 | 8.3 | 23 |
| Cyprididae sp. juveniles | | 103 | 75 | 16 |
| Copepoda | | | | |
| Calanoida sp. | | 1 | 8.3 | 23 |
| *Cyclops* sp. | | 1 | 8.3 | 23 |
| Cyclopoida sp. juveniles | | 5 | 25 | 17 |
| Oligochaeta | sp. | 16 | 25 | – |
| Nematoda | sp. | 15 | 41.6 | – |
| Rotifera | sp. | + | 41.6 | – |
| Acarina | sp. | 4 | 16.6 | – |
| Ciliata | sp. | + | 16.6 | – |

contrast only 12 species were identified to species level in the experimental hatchlings (Table 2). Taking into account the wide range of amount of sediment collected from waders (6.79–84.75 g), the number of individuals that hatched in the experiment is here presented as values standardized per gram of sediment, in order to determine the propagule pressure (Table 1). The estimated velocity of hatching (MVH) is also shown in Table 1. These variables give an idea of the potential magnitude of egg banks in such lakes.

Results on the hatching pattern, as indicated by ITH, showed that *Artemia parthenogenetica* Bowen and Sterling 1978, appeared mainly at day 8 followed by the rest of large branchiopods (anostracans and notostracans) (Table 2). One week later, we found ostracods, cyclopid copepods, and most cladoceran species. *Daphnia mediterranea* Alonso 1985, *Heterocypris barbara* (Gauthier and

Brehm 1928), calanoids, and *Cyclops* sp. were the taxa that needed longer incubation time at these conditions for hatching.

Figure 3 shows a huge variability in the hatching pattern for all taxa among samples. Nevertheless, a pattern close to a unimodal distribution is observed for some taxonomic groups through time during the first two weeks; Ostracoda, Anostraca, and Cladocera hatched sequentially in a similar pattern up to day 16 (Fig. 3). Copepoda and Notostraca, present in lower abundances, did not show such distribution pattern through time. Despite this initial temporal pattern, ostracods, cladocerans, and copepods increased to the highest abundances later on day 23; notably pushing ITH to higher values (Table 2).

Cumulative taxa richness increased throughout the experiment, notably after day 12 (Fig. 3). This day
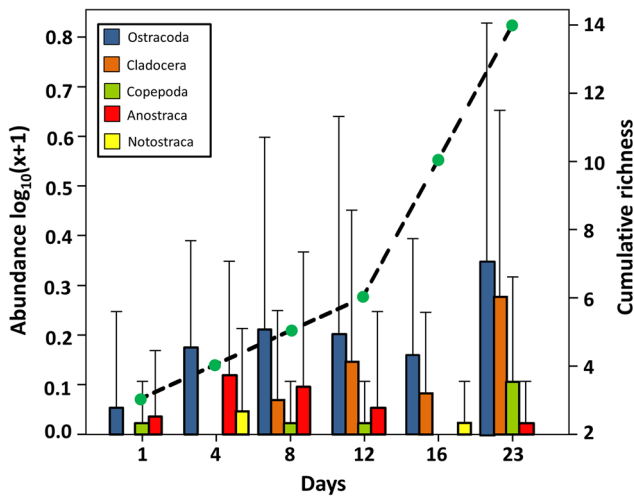
Fig. 3 Median and standard deviations of hatchlings abundance ($\log_{10}(x + 1)$)) during the experiment for each taxonomic group. Cumulative richness is represented with a *dashed line*
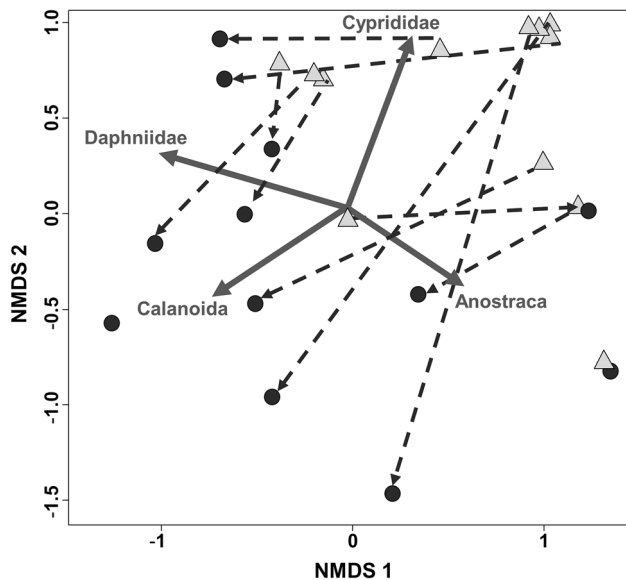


Fig. 4 NMDS ordination of crustaceans hatched in the experiment using the data in the (coarse resolution) taxonomic range of juveniles. *Dot symbols* represent active communities sampled directly in the lake and triangles communities hatched in aquaria from boots mud. *Dashed arrows* connect each boot sample with its respective lake sample. Significant taxonomic groups ($p < 0.05$) are represented with *arrows* in the plot. See text for further explanation
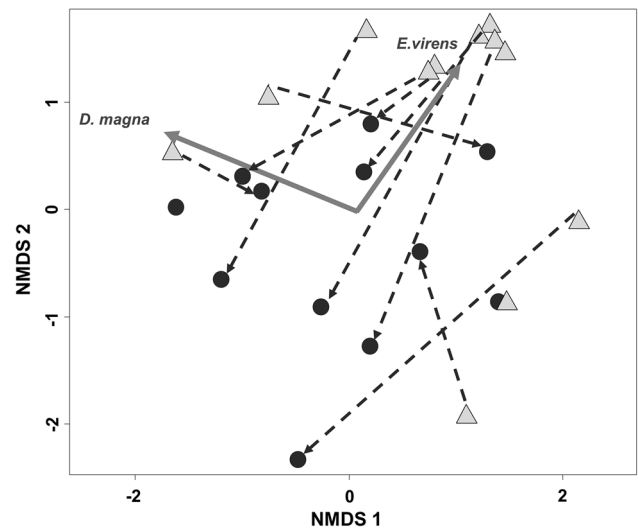


Fig. 5 NMDS ordination of crustaceans hatched in the experiment using data at species taxonomic level (i.e., excluding undetermined juveniles). *Dot symbols* represent active communities sampled directly in the lake and triangles communities hatched in aquaria from boots mud. *Dashed arrows* connect each boot sample with its respective lake sample. Significant taxonomic groups ($p < 0.05$) are represented with *arrows* in the plot. See text for further explanation

corresponds to a hatching maximum of cladoceran species. Eventually, 14 taxonomic groups were recovered, but this is probably an underestimation considering that many juveniles could not be identified to species level. Indeed, Ciliata, Oligochaeta, Nematoda, Rotifera, and Acarina were not identified to species and were not properly sampled due to the mesh size, but they could notably increase the total species richness obtained in the experiment.

The PERMANOVA analysis revealed significant differences ($p < 0.05$, $F$ value 3.91, $R^2$ 0.15) between the dispersed and active metacommunities. The ordination of high level taxa shown by the NMDS that included juveniles (Fig. 4), together with SIMPER results, showed that the taxonomic groups that contributed most to differences between the two sets of samples were Cyprididae (10.42 %), Daphniidae (9.86 %), Anostraca (9.05 %), and Calanoida (8.96 %). Cyprididae, the most abundant taxa hatched in the lab, was the only group with significant higher representation in the boots sediment samples hatched in the lab, and Daphniidae was the taxa encountered more frequently in field lake samples (mainly at low conductivities). Hellinger distances between lake source active communities and the corresponding dispersed communities (Fig. 4) showed a negative correlation with the conductivity of lakes ($p < 0.05$, $r = -0.79$). Therefore, lakes with high conductivity, which also present low species richness, showed the lowest distance values between their active and dispersed metacommunities.

Focusing on a higher taxonomic resolution but excluding juveniles, the NMDS obtained using species level identifications (Fig. 5) presented two species that significantly differed in relative abundance comparing active lake samples and their corresponding experimental communities hatched from boots mud: *Daphnia magna* Straus 1820, which predominated in field samples from the active metacommunity and *Eucypris virens* (Jurine 1820), mainly dominant in the dispersed communities hatched in the lab

from boots sediment. These two cosmopolitan and eury-topic species contributed together to 15 % of community differentiation according to SIMPER analysis. Other species differing in importance between both groups of samples were *Macrothrix hirsuticornis* Norman and Brady 1867, *Pleuroxus letourneuxi* (Richard 1888), and *A. parthenogenetica*. Lakes with high conductivity and presence of anostracans, either found in the active communities or in the experiments, ordered to the right of the NMDS graph (Fig. 4). The rest of samples presented a wide range of conductivities and communities mainly dominated by cladocerans and ostracods.

## DISCUSSION

Tourism, scientific research, and recreational activities are considered major vectors for human-mediated dispersal in protected lands (Pickering and Mount 2010). In the present survey, we demonstrate the effective potential of such kind of vectors in connecting wetland habitats. Nineteen invertebrate taxa hatched from mud attached to waders used in a set of protected lakes (Table 2), and probably a higher number of species might be expected taking into account the juveniles not identified to species level. In addition, propagules that did not hatch from the sediment during the duration of the experiment might also increment the propagule pressure related to these vectors.

In the present survey, we found higher richness of invertebrate taxa hatching from boots mud (including copepod and notostracan species) than in similar studies (Waterkeyn et al. 2010a), although this variation depends on the taxonomical resolution attained in the survey. These authors also found ostracods and cladocerans with high prevalence in waterproof boots. But why did we find in our experiments only 26.47 % of the invertebrate species found in the field samples? According to Waterkeyn et al. (2010b), lake salinity affects hatching patterns, survival, reproduction, and development of individuals hatched. Specific richness in most taxonomic groups is negatively affected by salinity (e.g., Boix et al. 2008), and this condition has a major effect on cladocerans (e.g., Jeppesen et al. 1994). In addition, the temporal habitat selection hypothesis suggests that species can adjust the timing for hatching depending on the environmental conditions (Vanoverbeke 2000). Probably, both reasons could explain the absence or the low hatchlings in mud from lakes with high salinity (e.g., Yeguas and Quero), also considering that the field measures of conductivity in these water bodies were very high (around 100 mS cm$^{-1}$). Moreover, the inter- and intra-annual variability of active communities and their production of resting eggs might also affect discrepancies between active and egg bank communities,

together with other environmental factors affecting community development (Waterkeyn et al. 2010b). In our study, notostracans and anostracans were the groups that needed the shortest time to hatch on average, although one cyclopoid copepod and a few cypridid ostracods also hatched so early in the experiments. These groups of crustaceans are typical inhabitants of short-hydroperiod temporary waters (Alonso 1996; Meisch 2000), and some have very fast life cycles adapted to grow and lay eggs before a more complex food web, rich in predating organisms, develops and drastically affects their population densities (Anderson et al. 1999; Lahr et al. 1999).

Human activities associated to water bodies are common in nature conservation areas. Invertebrate dispersal by human vectors in protected lakes, as reflected in this study, can have an important role in metacommunity structure (Waterkeyn et al. 2010a). Pristine conditions could be disturbed by such propagule pressure and consequently promote new colonization events, directly affecting the regional and local species diversity (Mouquet and Loreau 2003). High dispersal rate decrease β-diversity and could increase, in some lakes, α-diversity. Consequently, the high number of individuals per gram of sediment that we found, the high variety of invertebrate taxa dispersed and the intensive presence of human activities associated with these protected lakes could be affecting not only local processes, but also at the regional scale.

In our survey, *Eucypris virens* and *Daphnia magna*, two cosmopolitan species found in a wide range of conditions (Alonso 1996; Meisch 2000), were the species showing the highest capacity to be dispersed by footwear. Some cryptic species of the ostracod *E. virens* species group can be considered endemic to Central Spain (Schmit et al. 2013), but others are known as exotic invasive species in Australia (Koenders et al. 2012). *D. magna* was the most abundant cladoceran and the first crustacean colonizing several recently restored temporary ponds in dune slacks of Spain (Olmo et al. 2012). *Daphnia lumholtzi*, a congeneric of *D. magna* from African and Asiatic lakes, is also an exotic invader in America (Havel et al. 1995), and American clones of *D. pulex* have been considered as exotic invaders in Africa (Mergeay et al. 2005). These examples highlight the high potential dispersal of the genus *Daphnia* and particularly of *D. magna*. The observation of hatched *Triops cancriformis* (Lamarck 1801) in our experiments demonstrated that it can also be dispersed by human vectors. This species can predate on zooplankton resting eggs (Waterkeyn et al. 2011), reflecting the negative effects that it could have if introduced to a new habitat. Indeed, even *T. cancriformis* which is native in the Iberian Peninsula, it is considered invasive in Japan (NIES 2015). Transport of these organisms may affect other taxonomic groups associated to these water bodies so as those in close areas with

high conservation value (e.g., Tablas de Daimiel and Ruidera lakes). Indeed, the study area of "La Mancha Húmeda" Biosphere Reserve fulfills an important role as a refuge and feeding ground for a number of birds in the migration period (Florín and Montes 1999; Gosálvez et al. 2012). Species composition and abundances shifts in the invertebrate metacommunities could modify the principal food resources for many waterbirds. And these, in turn, may also act as dispersal vectors not only for native and even endangered species of aquatic invertebrates present in the area (such as *Candelacypris aragonica*; see e.g., Roca et al. 2000), favoring their metapopulation dynamics, but also for exotic species, some of which are present in some of the study lakes (see below) (Amezaga et al. 2002).

The presence and potential spread of exotic invasive species is a key factor to take into consideration in conservation management. In recent times, the distribution ranges of alien invertebrates have greatly expanded in freshwater systems (Ricciardi 2015). *Candonocypris novaezelandiae* (Baird 1843) is an exotic ostracod recently discovered in Spain (Valls et al. 2014b) and in particular in one of our study systems, lake La Veguilla in La Mancha Biosphere Reserve (Supplementary material S1), so as in other European countries (Scharf et al. 2014). Despite this species did not hatch from any of the analyzed boot samples, this does not prevent that in the future *C. novaezelandiae* could be further dispersed by human activities. A higher intensity of these vectors, so as secondary transports (e.g., car, train, and plane), will increase the possibility of new colonization events. Especially so if we take into account not only local movements of people, but also international tourism (for instance, bird watchers), lake management actions, and scientific activities (Pickering and Mount 2010; Valls et al. 2014a).

Dispersal has also a significant role in the genetic structure of metapopulations. Immigration and emigration rates among lakes affect the intraspecific diversity in a metapopulation structure. Genetic diversity increases the possibilities of confronting environmental changes, avoiding extinction, and fuels speciation processes (Bilton et al. 2001). Therefore, dispersal mechanisms are essential for the structure of metapopulations and their possibilities to survive in these unstable systems (e.g., shallow temporary lakes). However, few studies have been carried out to understand the paper of human dispersal vectors in these processes. New methods to measure dispersal have been developed in recent years such as the use of isotopes or fluorescent nanoparticles as egg mimics (Carlo et al. 2009; Lard et al. 2010; Tesson and Edelaar 2013). These new approaches could help understanding transport among lakes, retention time of mud adhered to waterproof footwear when repeatedly used in different sites, effects of dry and wet conditions for different species, frequency of dispersal, etc.

A more predictive understanding of impacts by human-mediated dispersal in aquatic metacommunities should be considered in future studies, particularly focusing on wetland conservation areas (Muñoz 2010; Cristescu and Boyce 2013). Pollution, urban wastewater, and overexploitation of aquifers are the principal human activities usually under control for conservation management in protected wetlands. Attention must also turn to tourism, scientific, and recreational activities associated with water bodies, because food webs, dispersal rates, and invasive species distributions could be modified by human vectors related to those activities. Cleaning protocols for footwear and clothes after their use in water bodies should be established and distributed. For instance, recommendations and protocols reported for military equipment and personnel movements to prevent human-mediated dispersal of invasive species (e.g., Cofrancesco et al. 2007) should be taken into consideration in new conservation management strategies. Education campaigns for tourists, scientists, governments, and companies associated with activities in protected lakes should also be planned and implemented. Future strategies should be started as soon as possible in the light of what is coming down the line to prevent biotic homogenization.

## CONCLUSIONS

Human-mediated dispersal represents a vector with capacity to disperse a huge range of invertebrate groups. Aquatic invertebrates with diapausing eggs can be easily transported passively by footwear and they can later hatch in a new habitat, as demonstrated in our experimental work. Some species, such as the pioneer and cosmopolitan crustaceans *Eucypris virens* and *Daphnia magna* seem specially fitted for such colonization processes. The timing of hatching patterns under experimental conditions reflected different strategies between groups, with anostracan hatchlings concentrated during the first weeks. Human and other vectors transporting species among close lakes may induce biotic homogenization in these protected water bodies, and they might eventually favor larger scale invasion processes. Therefore, controlling human activities close to water bodies should be considered in conservation programs of aquatic biodiversity.

🦋 Springer

# REFERENCES

Alekseev, V.R., B.T. Destasio, and J. Gilbert. 2007. *Diapause in aquatic invertebrates*. New York: Springer.

Alonso, M. 1996. *Fauna Iberica 7: Crustacea, Branchiopoda*. Madrid: Museo Nacional de Ciencias Naturales (CSIC).

Amat, F., F. Hontoria, O. Ruiz, A.J. Green, M.I. Sanchéz, J. Figuerola, and F. Hortas. 2005. The American brine shrimp *Artemia franciscana* as an exotic invasive species in the Western Mediterranean. *Biological Invasions* 7: 37–47.

Amezaga, J.M., L. Santamaría, and A.J. Green. 2002. Biotic wetland connectivity-supporting a new approach for wetland policy. *Acta Oecologica* 23: 213–222.

Anderson, C.R., B.L. Peckarsky, and S.A. Wissinger. 1999. Tinajas of southeastern Utah: Invertebrate reproductive strategies and the habitat templet. In *Invertebrates in freshwater wetlands of North America: Ecology and management*, ed. D.P. Batzer, R.B. Rader, and S.A. Wissinger, 791–810. New York: Wiley.

Ansong, M., and C. Pickering. 2014. Weed seeds on clothing: A global review. *Journal of Environmental Management* 144: 203–211.

Armengol, J., M. Estrada, A. Guiset, R. Margalef, D. Planas, J. Toja, and F. Vallespinós. 1975. Observaciones limnológicas en las lagunas de La Mancha. *Boletín Estación Central de Ecología* 4: 11–27.

Auffret, A.G., J. Berg, and S.A. Cousins. 2014. The geography of human-mediated dispersal. *Diversity and Distributions* 20: 1450–1456.

Boix, D., S. Gascón, J. Sala, A. Badosa, S. Brucet, R. López-Flores, M. Martinoy, J. Gifre, et al. 2008. Patterns of composition and species richness of crustaceans and aquatic insects along environmental gradients in Mediterranean water bodies. *Hydrobiologia* 597: 53–69.

Bilton, D.T., J.R. Freeland, and B. Okamura. 2001. Dispersal in freshwater invertebrates. *Annual Review of Ecology and Systematics* 32: 159–181.

Cáceres, C.E., and N.G. Hairston Jr. 1998. Benthic-pelagic coupling in planktonic crustaceans: The role of benthos. In *Evolutionary and ecological aspects of crustacean diapause*, ed. L. Brendonck, L. De Meester, and N.G. Hairston Jr, 163–174. Stuttgart: E. Schweizerbartsche-Verlagsbuchhandlung.

Carlo, T.A., J.J. Tewksbury, and C. Martinez del Rio. 2009. A new method to track seed dispersal and recruitment using 15N isotope enrichment. *Ecology* 90: 3516–3525.

Carlton, J.T. 1999. The scale and ecological consequences of biological invasions in the world's oceans. In *Invasive species and biodiversity management*, ed. O.T. Sandlund, P.J. Schei, and Å. Viken, 195–212. Dordrecht: Kluwer Academic Publishers.

Cofrancesco Jr, A.F., D.R. Reaves, and D.E. Averett. 2007. *Transfer of invasive species associated with the movement of military equipment and personnel*. Vicksburg: US Army Engineer Research and Development Center.

Cristescu, B., and M.S. Boyce. 2013. Focusing ecological research for conservation. *Ambio* 43: 805–815.

Derricourt, R. 2005. Getting "Out of Africa": Sea crossings, land crossings and culture in the hominin migrations. *Journal of World Prehistory* 19: 119–132.

Dussart, B. 1967. Copepodes des eaux continentales d'Europe Occidentale. In *Copepodes des eaux continentales d'Europe Occidentale*. Paris: N. Boubée & Cie.

Escrivà, A., R.J. Smith, J.A. Aguilar-Alberola, T. Kamiya, I. Karanovic, J. Rueda, E.I. Schornikov, and F. Mesquita-Joanes. 2012. Global distribution of *Fabaeformiscandona subacuta*: An exotic invasive Ostracoda on the Iberian Peninsula? *Journal of Crustacean Biology* 32: 949–961.

Florín, M., and C. Montes. 1999. Functional analysis and restoration of Mediterranean lagunas in the Mancha Húmeda Biosphere Reserve (Central Spain). *Aquatic Conservation: Marine and Freshwater Ecosystems* 9: 97–109.

Gosálvez, R.U., J.A. Gil-Delgado, C. Vives-Ferrándiz, E.G. Sánchez, and B.M. Florín. 2012. Monitoring threatened waterbirds in the lakes of the Biosphere Reserve "La Mancha Húmeda" (Central Spain). *POLÍGONOS. Revista de Geografía* 22: 89–122. **(In Spanish)**.

Griffiths, R.W., D.W. Schloesser, J.H. Leach, and W.P. Kovalak. 1991. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes region. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 1381–1388.

Hairston Jr, N.G., R.A. Van Brunt, C.M. Kearns, and D.R. Engstrom. 1995. Age and survivorship of diapausing eggs in a sediment egg bank. *Ecology* 76: 1706–1711.

Havel, J.E., W.R. Mabee, and J.R. Jones. 1995. Invasion of the exotic cladoceran *Daphnia lumholtzi* into North American reservoirs. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 151–160.

Oksanen, J., F.G. Blanchet, R. Kindt, P. Legendre, P.R. Minchin, R.B. O'Hara, G.L. Simpson, P. Solymos, et al. 2015. Vegan: Community ecology package. R package version 2.2-1. http://CRAN.R-project.org/package=vegan.

Jeppesen, E., M. Søndergaard, E. Kanstrup, B. Petersen, R.B. Eriksen, M. Hammershøj, E. Mortensen, J.P. Jensen, et al. 1994. Does the impact of nutrients on the biological structure and function of brackish and freshwater lakes differ? *Hydrobiologia* 275: 15–30.

Koenders, A., K. Martens, S. Halse, and I. Schön. 2012. Cryptic species of the *Eucypris virens* complex (Ostracoda, Crustacea) from Europe have invaded Western Australia. *Biological Invasions* 14: 2187–2201.

Korkmaz, S., D. Goksuluk, and G. Zararsiz. 2014. MVN: An R package for assessing multivariate normality. *The R Journal* 6: 151–163.

Lard, M., J. Bäckman, M. Yakovleva, B. Danielsson, and L.-A. Hansson. 2010. Tracking the small with the smallest-using nanotechnology in tracking zooplankton. *PLoS One* 5: e13516.

Lahr, J., A.O. Diallo, K.B. Ndour, A. Badji, and P.S. Diouf. 1999. Phenology of invertebrates living in a sahelian temporary pond. *Hydrobiologia* 405: 189–205.

Lenda, M., P. Skórka, J.M.H. Knops, D. Moron, W.J. Sutherland, K. Kuszewska, and M. Woyciechowski. 2014. Effect of the internet commerce on dispersal modes of invasive alien species. *PLoS One* 9: e99786.

Meisch, C. 2000. *Freshwater Ostracoda of western and central Europe*. Heidelberg: Spektrum Akademischer Verlag.

Mergeay, J., D. Verschuren, and L. De Meester. 2005. Cryptic invasion and dispersal of an American *Daphnia* in East Africa. *Limnology and Oceanography* 50: 1278–1283.

Mills, E.L., J.H. Leach, J.T. Carlton, and C.I. Secor. 1994. Exotic species and the integrity of the Great Lakes. *BioScience* 28: 767–771.

Mouquet, N., and M. Loreau. 2003. Community patterns in source-sink metacommunities. *American Naturalist* 162: 544–557.

Muñoz, J. 2010. Diversity and distribution of diapausing aquatic invertebrates in inland wetlands: An ecosystem conservation viewpoint. *Journal for Nature Conservation* 18: 55–62.

NIES. 2015. *Triops* spp. In *Invasive species of Japan*. Tsukuba: Invasive Species Research Team, Environmental Risk Research Center, National Institute for Environmental Studies. http://www.nies.go.jp/biodiversity/invasive/DB/detail/70400e.html.

Olmo, C., X. Armengol, and R. Ortells. 2012. Re-establishment of zooplankton communities in temporary ponds after autumn flooding: Does restoration age matter? *Limnologica* 42: 310–319.

Pickering, C.M., and A. Mount. 2010. Do tourists disperse weed seed? Vehicles and horses. *Journal of Sustainable Tourism* 18: 239–256.

Pickering, C.M., A. Mount, M.C. Wichmann, and J.M. Bullock. 2011. Estimating human-mediated dispersal of seeds within an Australian protected area. *Biological Invasions* 13: 1869–1880.

R Development Core Team. 2013. *A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. http://www.R-project.org.

Reichard, S.H., and P. White. 2001. Horticulture as a pathway of invasive plant introductions in the United States. *BioScience* 51: 103–113.

Ricciardi, A. 2015. Ecology of invasive alien invertebrates. In *Ecology and general biology: Freshwater invertebrates*, ed. J. Thorp, and D.C. Rogers, 83–91. London: Elsevier Inc.

Roberts, D.W. 2013. Labdsv: Ordination and multivariate analysis for ecology. R package version 1.6-1. http://CRAN.R-project.org/package=labdsv.

Roca, J.R., F. Mezquita, J. Rueda, A. Camacho, and M.R. Miracle. 2000. Endorheic versus karstic lakes: Patterns of ostracod distributions and lake typology in a Mediterranean landscape (Castilla—La Mancha, Spain). *Marine & Freshwater Research* 51: 311–319.

Scharf, B., C. Meisch, I. Schön, and K. Martens. 2014. New records of *Candonocypris novaezelandiae* (Crustacea, Ostracoda) from Germany, Belgium, England and Tunisia. *Abhandlungen des Naturwissenschaftlichen Vereins zu Bremen* 47: 337–344.

Schmit, O., I. Fukova, J. Vandekerkhove, Y. Michalakis, R. Matzke-Karasz, G. Rossetti, K. Martens, and F. Mesquita-Joanes. 2013. Mate recognition as a reproductive barrier in sexual and parthenogenetic *Eucypris virens* (Crustacea, Ostracoda). *Animal Behaviour* 85: 977–985.

Schmit, O., G. Rossetti, J. Vandekerkhove, and F. Mesquita. 2007. Food selection in *Eucypris virens* (Crustacea: Ostracoda) under experimental conditions. *Hydrobiologia* 585: 135–140.

Strasser, M. 1999. *Mya arenaria*—An ancient invader of the North Sea coast. *Helgoländer Meeresuntersuchungen* 52: 309–324.

Tesson, S.V.M., and P. Edelaar. 2013. Dispersal in a changing world: Opportunities, insights and challenges. *Movement Ecology* 1: 1–6.

Valls, L., A. Mestre, J.A. Gil-Delgado, and F. Mesquita-Joanes. 2014a. The shoemaker's son always goes barefoot: Intercontinental dispersal of Ostracoda (Crustacea) by scientists attending an IBS excursion. *Frontiers of Biogeography* 6: 89–91.

Valls, L., J. Rueda, and F. Mesquita-Joanes. 2014b. Rice fields as facilitators of freshwater invasions in protected wetlands: The case of Ostracoda (Crustacea) in the Albufera Natural Park (E Spain). *Zoological Studies* 53: 1–10.

Vandekerkhove, J., B. Niessen, S. Declerck, E. Jeppesen, J.M. Porcuna, L. Brendonck, and L. De Meester. 2004. Hatching rate and hatching success with and without isolation of zooplankton resting stages. *Hydrobiologia* 526: 235–241.

Vanoverbeke, J. 2000. Clones, sex and resting eggs: Temporal habitat selection and the genetic structure of cyclical parthenogenetic *Daphnia* populations. PhD Thesis, Katholieke Universiteit Leuven, Belgium.

Waterkeyn, A., B. Vanschoenwinkel, S. Elsen, M. Anton-Pardo, P. Grillas, and L. Brendonck. 2010a. Unintentional dispersal of aquatic invertebrates via footwear and motor vehicles in a Mediterranean wetland area. *Aquatic Conservation: Marine and Freshwater Ecosystems* 20: 580–587.

Waterkeyn, A., B. Vanschoenwinkel, P. Grillas, and L. Brendonck. 2010b. Effect of salinity on seasonal community patterns of Mediterranean temporary wetland crustaceans: a mesocosm study. *Limnology and Oceanography* 55: 1712–1722.

Waterkeyn, A., J. Vanoverbeke, N. Van Pottelbergh, and L. Brendonck. 2011. While they were sleeping: Dormant egg predation by *Triops*. *Journal of Plankton Research* 33: 1617–1621.

Worboys, G., T. DeLacy, and M. Lockwood. 2005. *Protected area management: Principles and practice*, 2nd ed. Cambridge: Cambridge University Press.

## AUTHOR BIOGRAPHIES

**Luis Valls** (✉) is a predoctoral student at the Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Paterna, Spain. His research interests include exotic species, human-mediated dispersal and metacommunity analysis.
*Address:* Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Paterna, Spain.
e-mail: luis.valls@uv.es

**Andreu Castillo-Escrivà** is a predoctoral student at the Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Paterna, Spain. His research interests include spatial analysis and genetic structure of ostracod metacommunities.
*Address:* Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Paterna, Spain.
e-mail: andreu.castillo@uv.es

**Francesc Mesquita-Joanes** is an Associate Professor at the Dept. of Microbiology and Ecology and Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain. His research interests include paleoecological analysis, exotic species, and (meta-) community ecology of Ostracoda (Crustacea).
*Address:* Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Paterna, Spain.
e-mail: mezquita@uv.es

**Xavier Armengol** is an Associate Professor at the Dept. of Microbiology and Ecology and Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Spain. His research interests include zooplankton ecology, paleoecological analysis, egg bank structure, and molecular ecology of zooplankton communities (crustaceans and rotifers).
*Address:* Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Paterna, Spain.
e-mail: javier.armengol@uv.es

🖄 Springer