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Dynamics of Ostracoda (Crustacea) assemblages in a Mediterranean pond system (Racó de l'Olla, Albufera Nat. Park) with focus on the exotic species *Candonocypris novaezelandiae* (Baird, 1843)

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Abstract – Coastal pond systems can account for a rich aquatic faunal diversity and complex functioning due to interplay between freshwater wetlands and marine influences. Within the large Albufera wetland Natural Park, Racó de l'Olla is constituted by a set of permanent and temporary ponds with strict protection level for migrating bird conservation. Its terrestrial faunal diversity and dynamics has been partially characterized in previous studies, but nothing was known about its ostracod communities. Benthic samples collected monthly for 1 year in 11 points through the pond system, together with limnological information, allowed establishing a sound knowledge on its ostracod community and population ecology. The most common species found were the euryhaline *Cyprideis torosa* and *Heterocypris salina*. In addition, it was remarkable the finding of a well-established population of *Candonocypris novaezelandiae*, a new finding for Europe and considered a potential alien invader. We used multivariate ordination and classification (PCA, RDA and TWINSPAN) to characterize the ostracod biocoenoses and taphocoenoses and their relation to the environmental variables. Our results suggest that hydroperiod and salinity are the main factors structuring ostracod communities in this system. Finally, we discuss the distribution and morphological aspects of *C. novaezelandiae* and its relation with passive dispersal in such a wetland with a dense population of migrating birds.

Key words: Population ecology / *Candonocypris novaezelandiae* / ostracods / Iberian Peninsula / alien species

Introduction

The introduction of alien species affects both the structure and the functioning of ecosystems by altering processes such as energy flows or by displacing native species from their ecological niche (Herbold and Moyle, 1986; Williamson, 1996). This problem has been rated as the second in importance in the list of threats to the biodiversity worldwide, having increased impacts on aquatic ecosystems (Mooney and Cleland, 2001). Recent research on the passive dispersal of invertebrates (Figuerola and Green, 2002) shows that, in addition to humans, birds can be one of the most important vectors for the dispersal of exotic aquatic invertebrates such as cladocerans, copepods and bryozoans (Leppäkoski *et al.*, 2002) along large distances (Green and Figuerola, 2005).

The passive dispersal of freshwater ostracods can be carried out by wind (Vanschoenwinkel *et al.*, 2007) and animals, including not only birds but also mammals (Vanschoenwinkel *et al.*, 2008), amphibians (Sabagh *et al.*, 2011), reptiles (Serramo *et al.*, 1999) and even other crustaceans (Aguilar-Alberola *et al.*, 2012). McKenzie and Hussainy (1968) already discussed how disjunct ostracod distributions might have originated from continental drift, but also from passive transport by birds. The retention time of propagules by birds (Figuerola and Green, 2002) has been calculated as of up to 72 h in *Anas discors* Linnaeus, 1766 a time enough for a similar species *Anas acuta* Linnaeus, 1758 to travel for about 1000 km (Miller *et al.*, 2003). Therefore, these types of vectors are of great importance to investigate how allochtonous ostracods colonize coastal Mediterranean wetlands, where the density of long-distance migrants can be much higher than in other regions (Green and Figuerola, 2005). However, dispersal is not the only factor that determines

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*Corresponding author: `luis5@alumni.uv.es` the distribution and abundance of species. Once organisms have arrived to a particular wetland, the major variables influencing the colonization process are the habitat characteristics that can affect the structure of benthic ostracod communities (Poquet *et al.*, 2008; Allen and Dodson, 2011). Eutrophication and salinity changes may influence community dynamics (Wetzel, 1983; Hargrave, 1991) and particularly ostracod species composition (Mesquita-Joanes *et al.*, 2012). In addition, these habitats present high inter- and intra-annual variability of their environmental conditions. Evaluation of these circumstances and factors are essential for a better understanding of the function of aquatic ecosystems.

The principal objective of our study was to assess the diversity of ostracods in the Racó de l'Olla Reserve, with particular focus on the exotic species that have colonized the area, probably via passive dispersal by aquatic birds. Moreover, we want to evaluate the effects of habitat characteristics on the distribution and abundance of the predominant species, so as the influence of temporary changes in the environment on population dynamics and the structure of ostracod communities. Anthropogenic impacts produce a drastic reduction in the ostracod biodiversity of wetlands (Poquet *et al.*, 2008). These changes can be produced mainly by the variation of salinity due to the human control of hydrological dynamics and by eutrophication (Altinsaçli and Mezquita, 2008; Poquet *et al.*, 2008). Thus, we want to emphasize the role of ostracods as sentinels of human impacts in aquatic ecosystems.

Materials and methods

The study area is located in the Nature Reserve Racó de l'Olla (UTM: 30S 0731620/4356742), a special protection zone included in the Albufera of Valencia Natural Park (Eastern Iberian Peninsula) and covers 48 ha (Fig. 1). During the first half of the 20th century human impact was intense (mainly through hunting and equitation activities), but for the last decades the Valencian government started a restoration plan and declared it a Special Integral Protection Reserve Area (Dies and Fernández, 1997), the highest protection level for an area inside such a Natural Park.

Currently, the Racó de l'Olla system is divided in four parts or ponds (Fig. 1). The western and eastern ponds, located in the central zone the Racó de l'Olla, are separated by a north–south sandbank. The southern pond is fed by water pumped from a canal connected to the Albufera Lake. This water is then diverted from here to the western or the eastern pond when they attain a low water level or to the reverse direction when the level is high. The remaining water comes from the water table and from the rain. Therefore, there are four zones with different characteristics: the northern and southern ponds are oligohaline water bodies, while the western and eastern ponds are mesohaline to polyhaline in their northernmost parts, and polyhaline to hyperhaline in their southernmost areas (Vicente *et al.*, 1996).

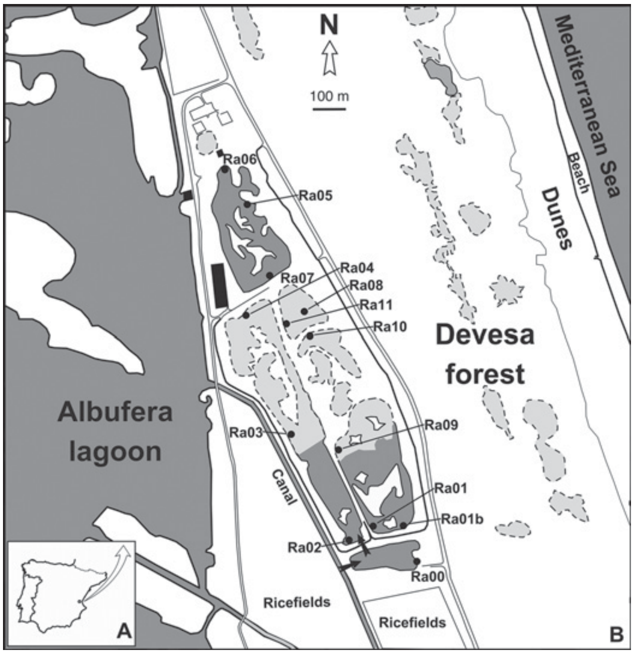


Fig. 1. Location of sampling sites in the Nature Reserve Racó de l'Olla in the Albufera of Valencia Natural Park (eastern Iberian Peninsula).

Monthly sampling surveys were carried out between January and December 2006 on 13 points in the Racó de l'Olla area (Fig. 1). At the same time, physicochemical environmental data and ostracod samples were obtained. Sample coding was composed of one number that represents the sampling month (1–12) plus the text "Ra" (as a reference to Racó de l'Olla), followed by the sampling site number (00–11, and 01b). Out of these 13 points we will not include point Ra01 in the analysis (but instead the adjacent one Ra01b), neither sites Ra05, Ra10 and Ra11 because we did not find ostracods in these sites, keeping therefore a total of nine sampling points for further analysis. In the field, we measured pH, oxygen content, electric conductivity, salinity and water temperature with portable probes. At the same time we collected water samples for subsequent analysis in the laboratory for alkalinity and chloride concentration. Benthic samples were taken with a 100 µm mesh size, D-frame hand net over an approximate area of 600 cm^2. The sediment obtained was fixed in the field with 30% ethanol. In the laboratory the sediment samples were washed and sieved through a 250 µm mesh sieve and stored in 70% ethanol. Ostracods were hand-picked under a stereomicroscope and stored in 70% ethanol for specific determinations. All complete animals living at the time of sampling (*i.e.*, belonging to the biocoenosis) were recovered, as well as the disarticulated valves and empty shells belonging to the taphocoenosis. Determinations of ostracods were carried out up to the species level, mainly using the taxonomic works by Meisch (2000), Athersuch (1982),

Table 1. Mean and standard deviation values of physicochemical variables analysed during the study period at different sampling points in Racó de l'Olla.

Sampling site	RA00	RA01b	RA02	RA03	RA04	RA06	RA07	RA08	RA09
N	218	61	414	141	101	401	578	3	9
n	12	9	12	8	7	8	12	1	6
Cond (mS.cm^{-1})	3.9 ± 0.8	31.3 ± 0.1	14.2 ± 0.7	20.7 ± 0.8	26.5 ± 1.0	18.7 ± 0.5	21.0 ± 0.7	4.1	28.6 ± 0.1
Sal (g.L^{-1})	2.1 ± 0.4	19.7 ± 10	8.3 ± 4.4	12.5 ± 4.8	16.4 ± 6.8	11.2 ± 3.4	12.7 ± 4.5	2.2	17.8 ± 4.6
O_2 (mg.L^{-1})	6.4 ± 3.1	7.4 ± 4.9	5.9 ± 3.3	5.0 ± 3	10.3 ± 4.7	7.6 ± 5.2	3.9 ± 3.2	7.4	8.5 ± 1.5
O_2 (%)	69 ± 31	75 ± 49	64 ± 39	53 ± 34	109 ± 58	84 ± 59	39 ± 30	66	86 ± 15
Temp (°C)	19.7 ± 7.3	17.3 ± 6.8	19.6 ± 6.9	17.0 ± 5.4	16.1 ± 5.6	17.3 ± 7.5	18.4 ± 6.1	9.9	14.1 ± 3.1
pH	7.6 ± 0.5	8.4 ± 0.4	7.9 ± 0.3	8.1 ± 0.2	8.5 ± 0.4	8.1 ± 0.4	8.0 ± 0.5	7.6	8.5 ± 0.2
Cl (mg.L^{-1})	18 ± 4	246 ± 123	93 ± 54	168 ± 57	195 ± 78	133 ± 37	150 ± 46	20.5	219 ± 54
Alk (mmol.L^{-1})	3.1 ± 1.1	5.1 ± 1.0	4.7 ± 1.1	5.7 ± 1.2	5.9 ± 0.8	6.1 ± 0.7	6.1 ± 1.2	3.2	5.6 ± 0.7
Alk/Cl	0.9 ± 2.0	0.7 ± 0.3	1.8 ± 1.9	1.2 ± 0.2	1.1 ± 0.3	1.6 ± 0.5	1.4 ± 0.4	5.5	0.9 ± 0.2
H'	4.3 ± 2.8	0.9 ± 1.6	3.1 ± 3.4	1.2 ± 0.6	0	1 ± 0.7	1.7 ± 1.4	0	0

N, total number of ostracods; *n*, number of samples; Cond, electrical conductivity; Sal, salinity; O_2, oxygen content; Temp, temperature; Alk, alkalinity; H', Shannon diversity index.

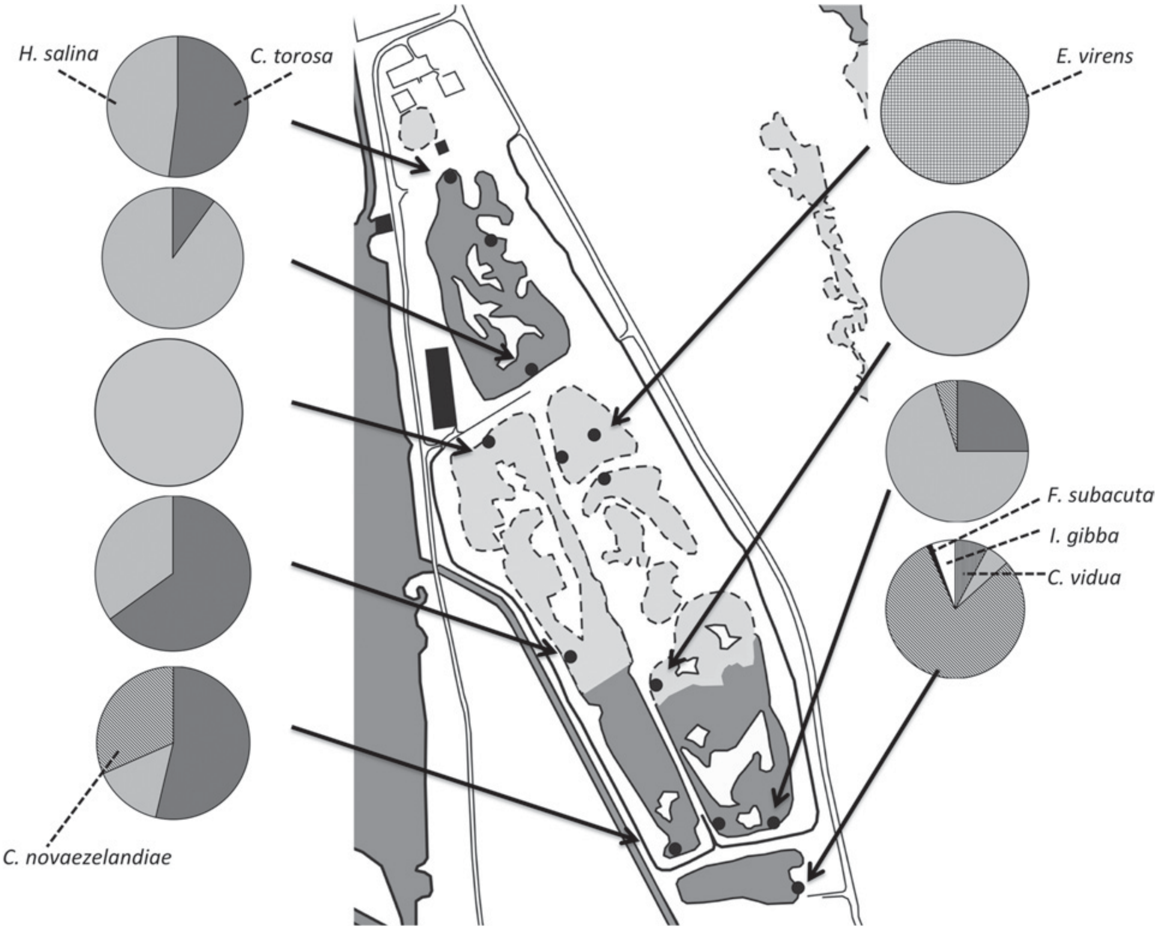


Fig. 2. Pie charts showing abundance percentages for the ostracod species found in the biocoenoses of each sampling station during the whole sampling period.

Janz (1994) and Eagar (1994). We obtained SEM photos of ostracod valves with a HITACHI S4800 microscope at the University of Valencia. We could not take pictures of *Eucypris virens* (Jurine, 1820) and *Fabaeformiscandona subacuta* (Yang, 1982) because of the poor preservation of their valves.

Owing to the high number of valves found in the taphocoenoses we used a scale of abundance instead of counting all the individuals as in the biocoenoses. The scale was coded as 1 = 1 individual; 2 = 2–5 individuals; 3 = 6–25 individuals; 4 = 26–125 individuals; 5 = more than 125 individuals. The same scale was applied

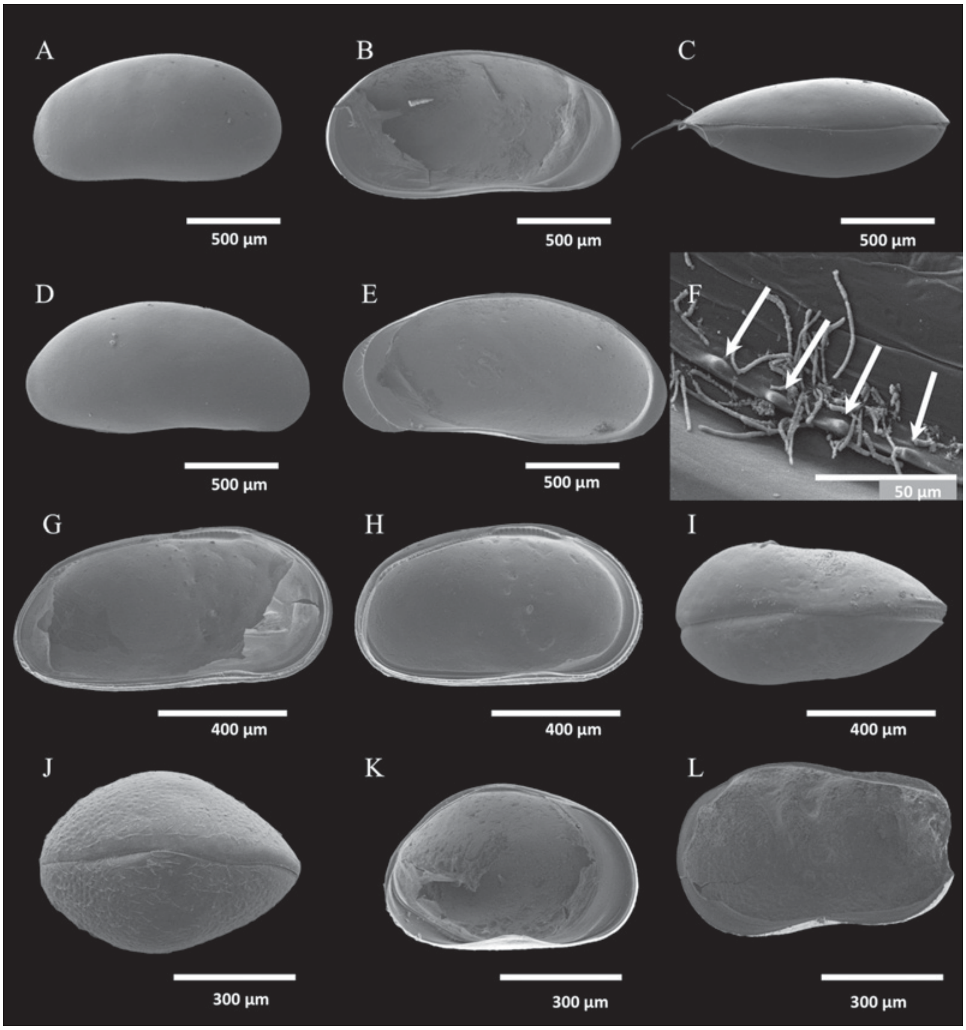


Fig. 3. SEM pictures of ostracod species found in Racó de l'Olla. A–F: *Candonocypris novaezelandiae* (Baird, 1843) A, external view of left valve (LV); B, inner view of LV; C, dorsal view; D, external view of right valve (RV); E, inner view RV; F, detail inner list LV with white arrows highlighting the pustules; G–I, *Cyprideis torosa* (Jones, 1850) G, male inner view LV; H, female inner view LV; I, female dorsal view; J–K, *Cypridopsis vidua* (OF Müller, 1776) J, dorsal view; K, inner view LV; L, *Ilyocypris gibba* (Ramdhor, 1808) inner view RV.

to the biocoenoses data on number of individuals accumulated through the year when comparing it with the taphocoenoses, to be able to carry out a comparative analysis (principal component analysis (PCA), see below) between past and present ostracod assemblages.

The software CANOCO (ter Braak and Šmilauer, 2002) was used for multivariate analyses, including PCA, detrended canonical correspondence analysis (DCCA) and redundancy analysis (RDA). In DCCA and RDA, electrical conductivity and dissolved oxygen were square root-transformed, the ratio alkalinity/chloride (concentrations in meq.L^{-1}) was $\log_{10}$ (square root)-transformed and the other variables were left untransformed because they did not differ significantly from a normal distribution. In RDA, Monte-Carlo's test was used to test for statistical significant relationships between the following variables and the structure of the living ostracod community: conductivity, temperature, pH, oxygen content and relation alkalinity/chloride. TWINSPAN (Hill, 1979) was used to analyse the biocoenoses data to classify samples according to the dominant species. In addition, we used the program C2 (Juggins, 2007) to obtain a coupled graph of the temporal dynamics for each species.

Results

Table 1 shows summarized abiotic and biotic information for the nine sampling stations monitored. They differed notably in water level variability and hydroperiod, what resulted in a variable number of samples (maximum of 12 monthly visits), depending on the presence or absence of free water. For example, the northern and western ponds were 37 cm deep in January and decreased progressively to values of 10 cm in the northern pond and 13 cm in the western pond in July. Sampling points with permanent water (Ra00 and Ra02) recorded lower values of conductivity, salinity, pH, chloride concentration and

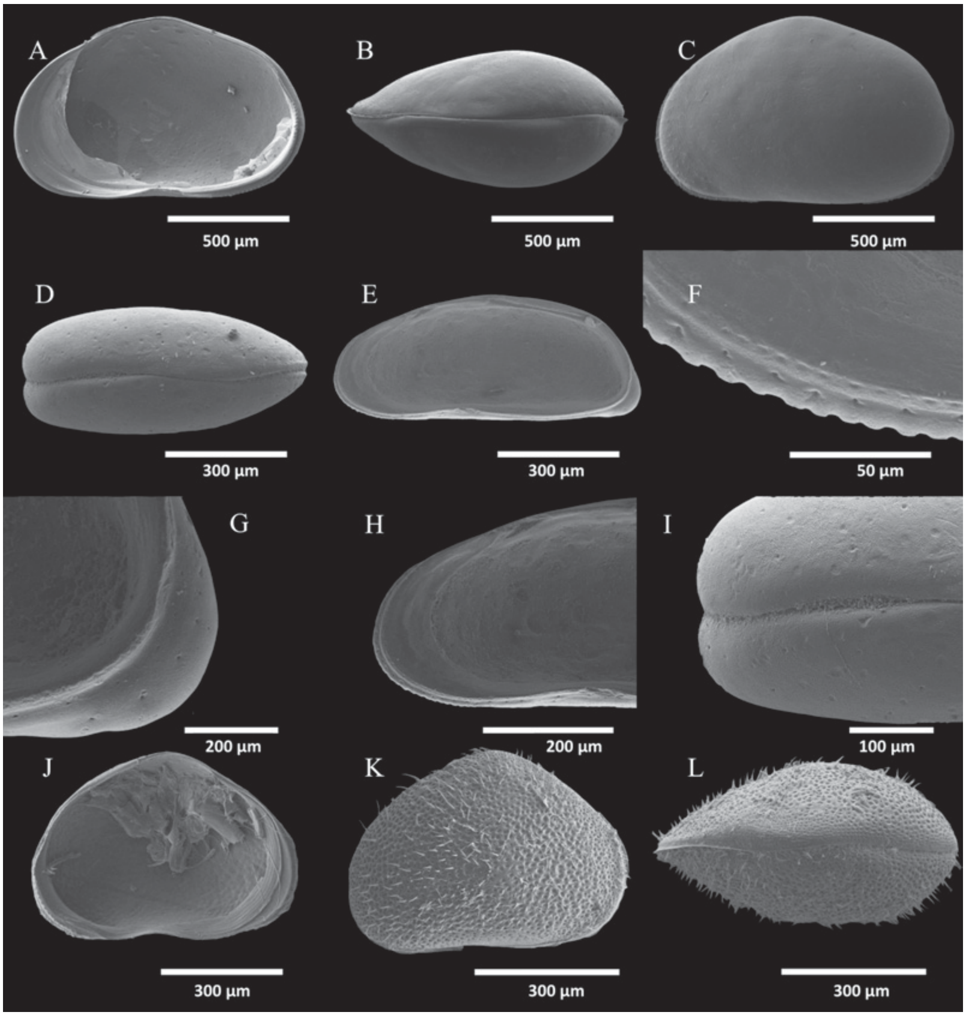


Fig. 4. SEM pictures of ostracod species found in Racó de l'Olla. A–C, *Heterocypris salina* (Brady, 1868) A, inner view RV; B, dorsal view; C, external view LV; D–I, *Pontocythere* cf. *rubra* (Müller, 1894) D, dorsal view; E, inner view RV; F, anterior detail inner view RV; G, posterior detail inner view RV; H, anterior detail inner view RV; I, posterodorsal detail of carapace; J–L, *Sarscypridopsis aculeata* (Costa, 1847) J, inner view LV; K, external view RV; L, dorsal view, carapace.

alkalinity than other points (except Ra08). In this last station (Ra08), we collected only one sample as a result of its short hydroperiod. There we measured a very low conductivity, due to rains during the previous days in January (AEMET, 2011).

Nine ostracod species were found during the whole study period (Figs. 2–4). Out of these, only seven were found alive in the biocoenoses (Fig. 2). The maximum diversity was measured in Ra00 and Ra02 (Table 1). In Ra00 we found the highest species richness, with a dominance of the exotic species *Candonocypris novaezelandiae* (Baird, 1843), together with *E. virens*, *Cypridopsis vidua* (OF Müller, 1776), *Ilyocypris gibba* (Ramdhor, 1808) and *F. subacuta*. In Ra01b and Ra02, we found three species in different proportions: *C. novaezelandiae*, *Heterocypris salina* (Brady, 1868) and *Cyprideis torosa* (Jones, 1850). In site Ra02, the dominant species were *C. torosa* (51.2%) and *C. novaezelandiae* (34.1%), whereas in sampling point Ra01b, characterized by higher conductivity, chloride content and pH, *H. salina* (70.5%) was the dominant species. In sites Ra03, Ra04, Ra06, Ra07 and Ra09, only two species appear: *H. salina* and *C. torosa*. The only sample collected in Ra08 harboured just three individuals of the species *E. virens*. The species *C. vidua*, *E. virens* and *F. subacuta* were not found in the taphocoenoses.

In the taphocoenoses, we found species that were not represented in the biocoenoses. These were *Sarscypridopsis aculeata* (Costa, 1847), collected in Ra03, Ra07 and Ra09, and *Pontocythere* cf. *rubra* (Müller, 1894). Figure 5 shows the PCA results comparing data from the biocoenoses with the taphocoenoses. This figure represents the ordination of samples along axis 1 (40.7%) and 2 (29.5%) of the PCA. In the negative extreme of both axes, we find species widely tolerant to salinity variations: *C. torosa*, *H. salina* and *S. aculeata*, together with the uncommon brackish species *P.* cf. *rubra*. Species *E. virens*, *C. novaezelandiae*, *C. vidua*, *F. subacuta* and *I. gibba*, with preferences for lower salinity conditions, were ordered on the positive part of the first axis. Therefore, axis 1 seems to be clearly

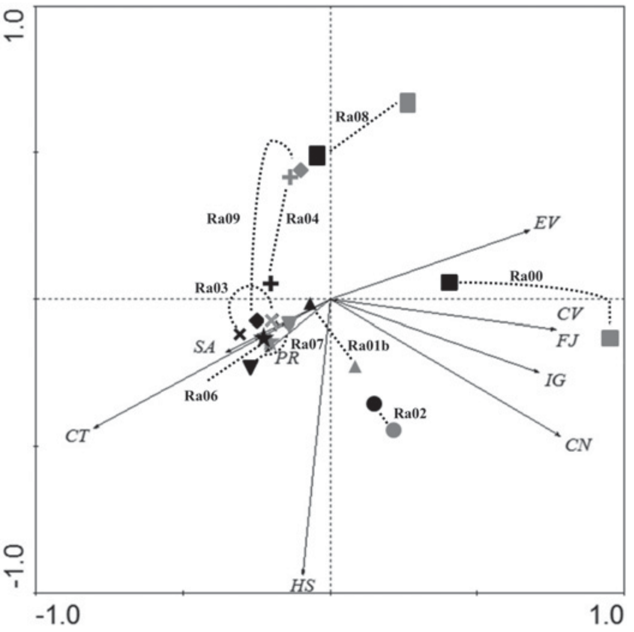


Fig. 5. Ordination graph of ostracod samples belonging to the taphocoenosis (black symbols) and the biocoenosis (grey symbols) on the first two axes of PCA. Dotted lines link biocoenoses and taphocoenoses of the same sampling site. Species codes: CT, *Cyprideis torosa*; HS, *Heterocypris salina*; SA, *Sarscypridopsis aculeata*; PR, *Pontocythere* cf. *rubra*; EV, *Eucypris virens*; CN, *Candonocypris novaezelandiae*; CV, *Cypridopsis vidua*; FJ, *Fabaeformiscandona subacuta*; IG, *Ilyocypris gibba*.

associated with salinity. As most sites display a more or less marked difference between their taphocoenoses and biocoenoses, and in all cases the change is towards most positive values of the first PCA axis, it could be interpreted as an indication of decreasing salinity from past to modern ostracod communities.

We found high densities of *C. torosa* along the year, showing the highest values in March (297 individuals) (Fig. 6), but ovigerous females were not found in late fall and winter months. As subdominant species we found *H. salina* and *C. novaezelandiae*. *H. salina* presented a peak of juvenile abundance in spring that soon moulted to adults. In the case of *C. novaezelandiae* we found two periods throughout the year where there was a high number of juveniles. The first juvenile cohort hatched in spring, and the second one in autumn. The species *C. vidua* appeared in late summer-early autumn to the end of the year, but at low densities.

The classification obtained with the TWINSPAN analysis up to the second level of division gives four groups of samples according to differences in species abundances (Fig. 7). Group I was composed of 27 samples belonging to sites Ra01b, Ra06, Ra07 and Ra02, where *H. salina* is the representative species. Group II encompasses 25 samples from sites that were absent in group I. Here, we find the highest densities of *C. torosa*. Group III has 17 samples from sites Ra00, Ra01b and Ra02, being *C. novaezelandiae* the representative species. Finally, group IV is made up of just two samples from sites Ra00 (where *C. novaezelandiae* is also found) and Ra08, its sole sample, where *E. virens* appeared in January. Four TWINSPAN groups were found in January (Fig. 8), when high ostracod diversity is detected. Sites in the northern part of the pond system (sampling points Ra06 and Ra07) were usually classified as belonging to groups I and II, being group I absent during the summer months. The central zone presents samples classified in any of the four TWINSPAN groups in January, but groups I and II dominate throughout the year (except in October, with only one site in group III). From May to October, many sampling sites in the central area dry up. From November on, these sites refill and mostly belong to group II. In the southern part, site Ra00 is classified in group III for most of the year, although no ostracods were captured alive in March and it was classified in group IV in January and in group II in June.

To better discern the relationship between the environmental variables and the ostracod living community we first carried out a DCCA with a total inertia of 1.991 and therefore, with such a short gradient, we chose to apply an RDA linear ordination method. The resulting ordination graph represented by axis 1 (14.6% of explained variance) and axis 2 (0.6% of variance) is shown in Fig. 9. The first axis is related to electrical conductivity, ordering samples and sites with higher values on its positive part and those with lower values on its negative side. Most ostracod species found (mainly collected in sites Ra00, Ra02 and Ra08), except *H. salina* and *C. torosa*, are ordered on the negative part of the first axis. The ordination of samples along axis 2 is related with water temperature, oxygen content and pH, showing the effects of seasonal changes in these variables (autumn and winter samples are mostly ordered on the negative part of this axis while spring and summer samples are situated on the opposite side).

Discussion

In general, many species are absent in many places where they could live, due to a combination of distance and unfavourable environmental conditions, making immigration and the establishment of new populations a low probability event (Lomolino *et al.*, 2010). Our study highlights the importance of the variation in conductivity (Table 1) in the system and, consequently, the aquatic environments studied present predominance of euryhaline species. Electric conductivity ranges from 4 mS.cm^{-1} in sites Ra00 and Ra08 to values around 20 mS.cm^{-1} in other stations, in agreement with previous data for the area (Soria and Ros, 1991; Vicente *et al.*, 1996). The large variation in the concentration of chloride and other ions produces physiological problems in osmoregulation, making these sites to constitute critical barriers for many aquatic species. The case of site Ra08 is different because the short hydroperiod of this point makes it suitable only for species such as *E. virens*, typical of temporary

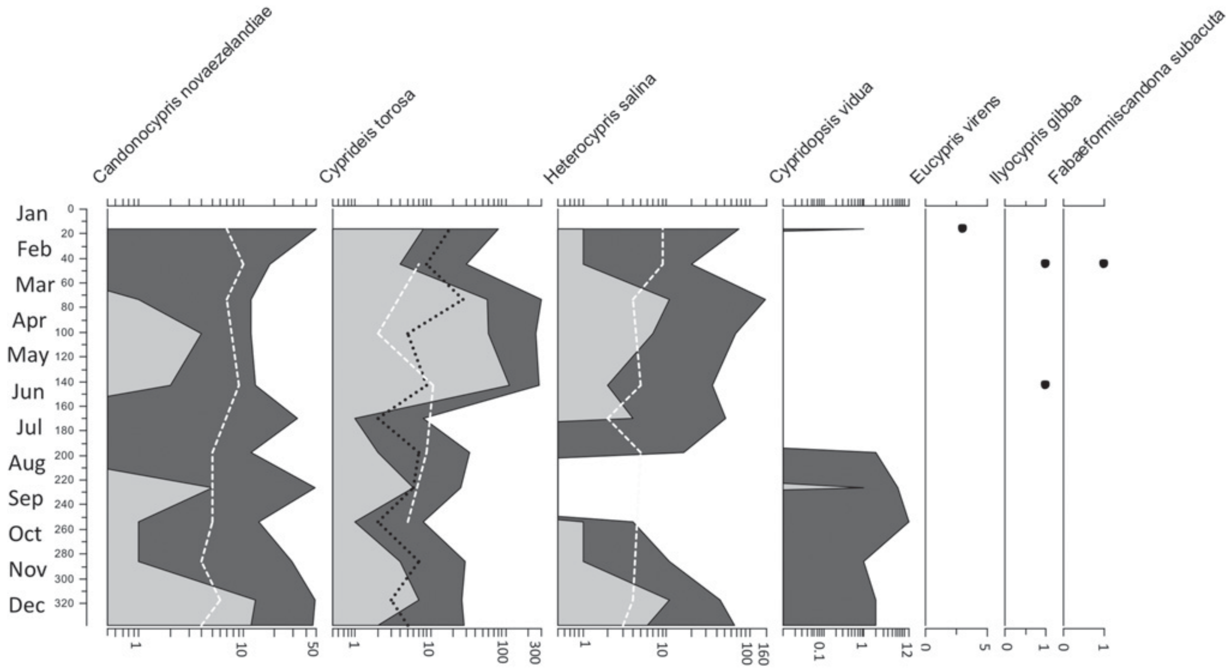
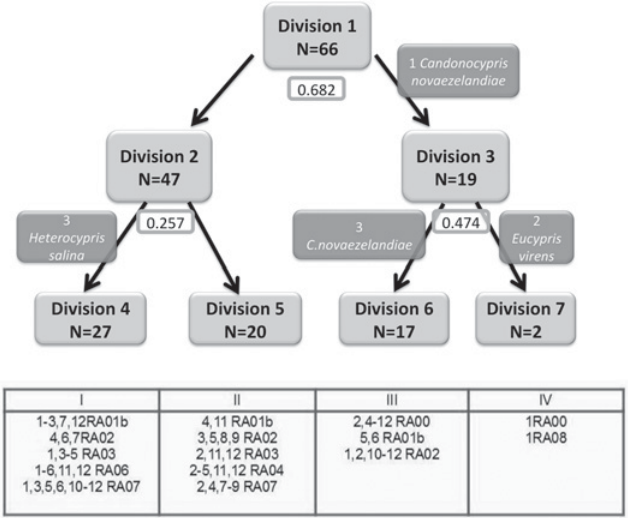


Fig. 6. Temporal dynamics of ostracod species in Racó de l'Olla. Abscissae: individuals per sample (axis in $\log_{10}$-scale, except for the last three species). Dark grey areas correspond to total species abundances (adults + juveniles); light grey areas correspond to number of juvenile individuals. Dotted black line represents male abundance. Dashed white line shows the average number of eggs per female. The total number of individuals for uncommon species is represented by black dots.



I	II	III	IV
1-3,7,12RA01b 4,6,7RA02 1,3-5 RA03 1-6,11,12 RA06 1,3,5,6,10-12 RA07	4,11 RA01b 3,5,8,9 RA02 2,11,12 RA03 2-5,11,12 RA04 2,4,7-9 RA07	2,4-12 RA00 5,6 RA01b 1,2,10-12 RA02	1RA00 1RA08

Fig. 7. Classification of samples obtained with TWINSPAN analysis. Indicator species and their level of abundance (1–3) are shown for each division. Eigenvalues for each division are also indicated in boxes.

freshwater environments through Europe and around the study area in particular (Meisch, 2000; Rueda *et al.*, 2006).

C. torosa, a euryhaline species that can live in hypersaline habitats (Meisch, 2000; Mezquita *et al.*, 2000) is one of the dominant ostracods of the studied metacommunity. Both Heip (1976) and Mezquita *et al.* (2000) found two generations per year of this species, and we corroborated this finding in our study. All the individuals that we found belonged to the morphotype with unnoded shells that is common at medium-to-high salinities. *H. salina* usually presents two or three generations for year (Meisch, 2000) and could disappear in November (leaving only diapausing eggs), according to Ganning (1971). In contrast, in our study, the disappearance of the population is observed in July, triggered by an important reduction of water level, but resting eggs survive the desiccation period and the population is later restored. In the taphocoenoses we found *S. aculeata* in all the ponds (except the southern one), with salinities of about 15 $\mathrm{g.L^{-1}}$, whereas the optimal values for the growth of this species are considered to be about 5–10 $\mathrm{g.L^{-1}}$ (Meisch, 2000). According to our PCA comparing the taphocoenoses with the biocoenoses, the past environmental conditions of the Racó de l'Olla could generally be characterized by higher levels of salinity in some points, which would not explain the presence of valves of *I. gibba* in Ra01b and high number of valves of *C. novaezelandiae* in Ra02 because they are species that do not tolerate high salinity (De Deckker, 1981; Meisch, 2000). However, Mediterranean coastal wetlands experience wide inter-annual variability of the environmental conditions (Poquet *et al.*, 2008; Waterkeyn *et al.*, 2008), which can trigger changes in the composition of communities, therefore allowing for some periods the growth of populations of these species.

One of the most particular species was found in the taphocoenosis of Ra07: *P.* cf. *rubra*. In the revision of this genus by Athersuch (1982) we could discern the morphological characteristics of the valve of this species. Our individuals measured 650 and 740 μm of length, in

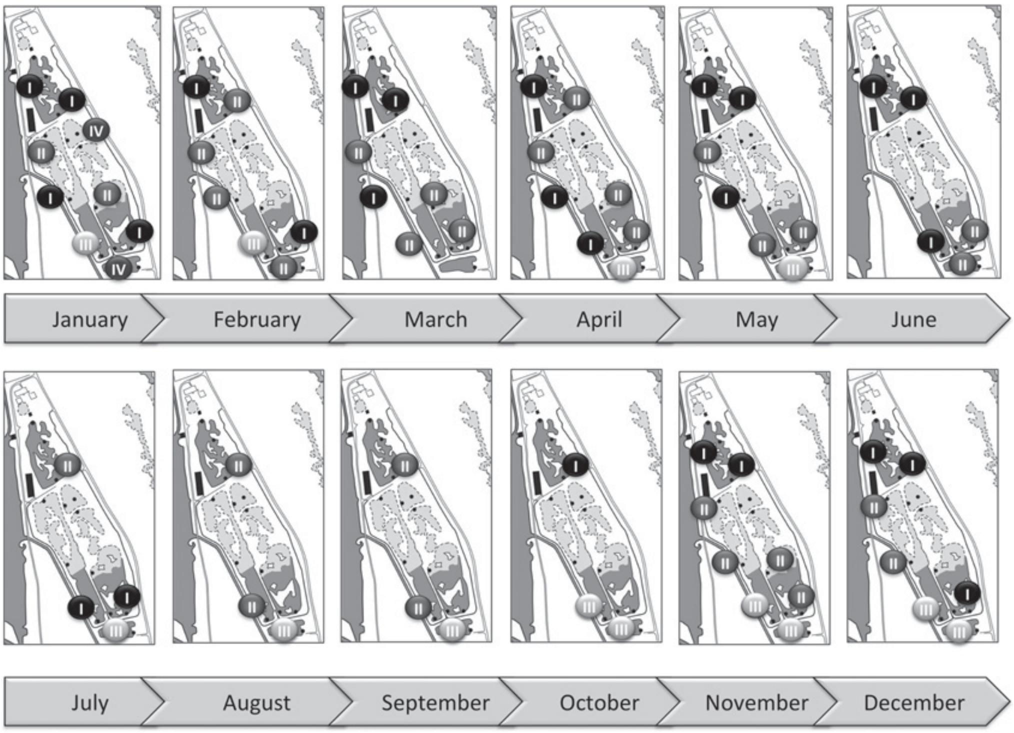


Fig. 8. Monthly variation of groups obtained by TWINSPAN analysis represented on a map of the sampling stations.

correspondence with the measures of the females of *P. rubra* that are 740 μm on average, whereas males are 875 μm long. The frontal part of the shell is elongated and gradually decreases towards the posterior part, whereas in *P. turbida* (with a similar length of *P. rubra*) it presents a gradually, less pronounced frontal part and a more orthogonal posterior part. In the isolated valve collected for this species, we found the presence of a row of small pustules of approximately 5 μm in size, distributed on the anteroventral margin of the right valve. The species has not been previously cited in the Valencian coast, but it is known from Algeria, Greece and the coasts of Italy (Adriatic and Tyrrhenian seas).

Finally, we want to focus on the most remarkable species in our study: *C. novaezelandiae*. Regarding morphological aspects, it is important to note that in the left valves of the *C. novaezelandiae* individuals figured previously in published articles we did not observe protuberances or any morphological variation similar to the one that we have found with the use of SEM on the inner part of the left valve. We obtained pictures of a row of small pustules located in the posteroventral inner list of the left valve, which measured 7.5 μm of length and were placed at a distance between them of about 15 μm (Fig. 3). This indicates either morphological differences between this and other populations or perhaps that this feature has remained unnoticed by previous authors.

C. novaezelandiae was the first ostracod species described from New Zealand (by William Baird, in White and Doubleday, 1843), from specimens collected by the naturalist-explorer Ernst Dieffenbach (Webber *et al*, 2010). As far as we know, this is the first time that the species is found in Europe. However, we can confirm that some undetermined Cyprididae species found in Vinaròs (Castelló province, about 140 km NE from our study area) by Escrivà (2011) and Mezquita *et al.* (2011) belong to the same species. The biogeography of this species encompasses the river Nile in Egypt (Ebtesam, 2010), South Africa (Sars, 1924; Martens, 1984; Martens *et al.*, 1998), Australia (De Deckker, 1981), China (Yu *et al.*, 2010), New Zealand (Eagar, 1994), New Caledonia (De Deckker, 1983) and Japan (Okubo, 1975). It is necessary to note that Ebtesam (2010) mentions its presence also in South America, citing Martens (1989) in support of it. However, Martens (1989) includes a figure of this species in a plate, but does not inform on the origin of the figured specimens. Martens (personal communication) kindly checked the collection code of these animals and informed us that they were from Australia. In general, the area of origin where it is considered autochthonous is Australasia, because males of the species can be found in the region (De Deckker, 1983) and the species is found there in Pleistocene deposits (Eagar, 1995), but never in European Quaternary sedimentary records (Griffiths, 1995). The area closest to Spain where individuals of *C. novaezelandiae* have been found is the river Nile in Egypt (Ebtesam, 2010), a zone that may have served as a stepping-stone for further colonization of Europe from Eastern Asia or Australasia. From northern Africa, the dispersal of this species north to our study area might have been facilitated by migrating birds, perhaps as resting eggs resisting their digestive system (Green and Figuerola, 2005), although the possibility that they arrived through passive dispersal by humans (*e.g.*, Fox, 1965;

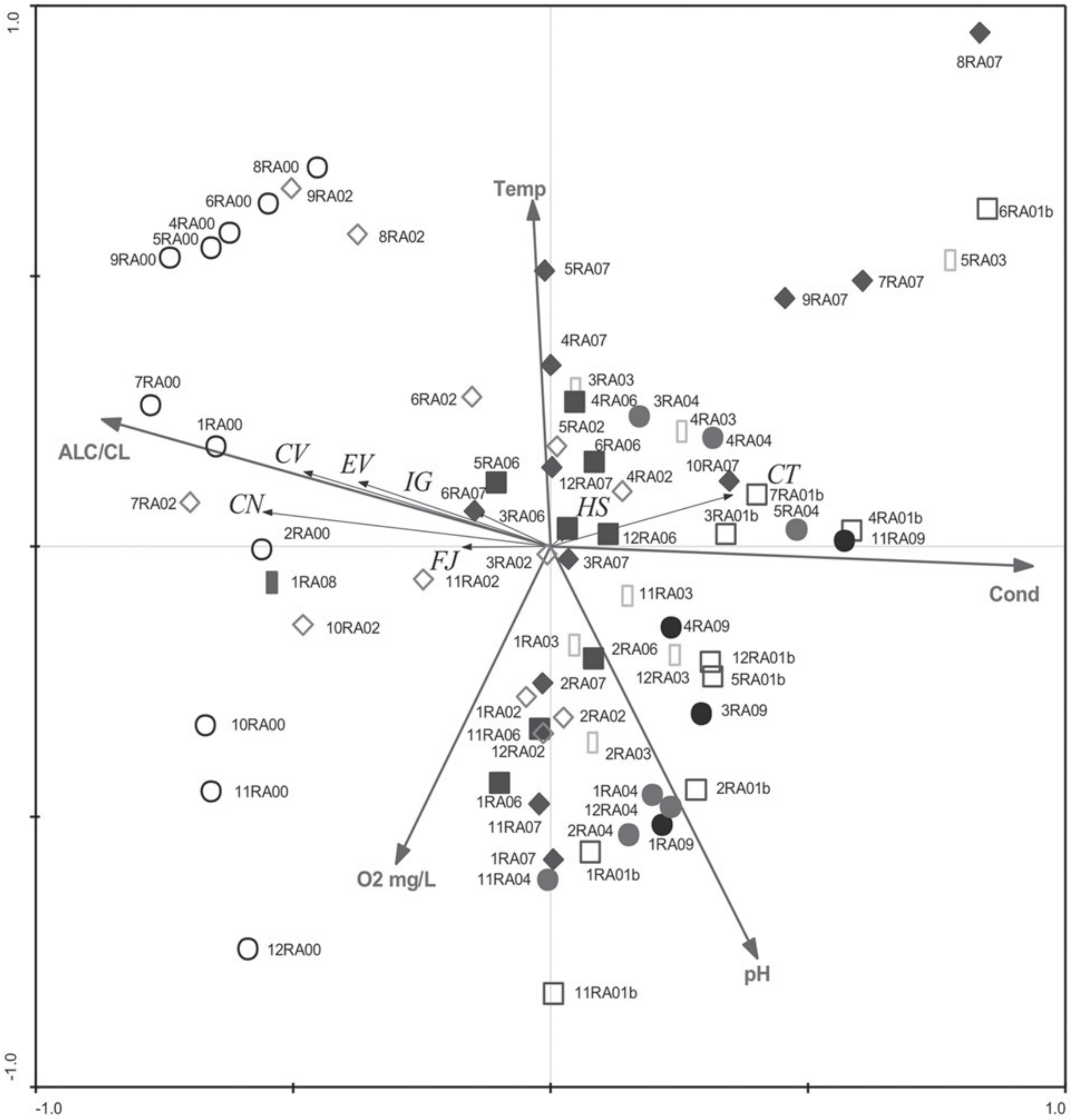


Fig. 9. Triplot graph showing the results of the RDA with species, samples and environmental variables measured in Racó de l'Olla. Different symbols correspond to each sampling station. Each sample is labelled with a numeric code (1–12) indicating the monthly period, along with the code of the sampling site. Species codes as in Figure 5. Environmental variables codes as in Table 1.

Waterkeyn *et al.*, 2010) cannot be discarded, and more information is needed as to whether or not the species has colonized other European regions as well.

In the TWINSPAN ecological groups obtained, *C. novaezealandiae* is the predominant species in group III, which makes us raise some questions: How was the ecosystem of the Racó de l'Olla before the appearance of this species? What effects has it had on the ecosystem? Is there a direct relation between the number of long-distance aquatic migratory birds and the presence of new citations of ostracods in the Iberian Peninsula and other areas? We can imagine that, in the same way as it will be more probable to find a coin of another country in an airport where high number of planes arrive from remote regions than in those airports that only receive a small number of flights from nearby places, the aquatic ecosystems with a high number of aquatic migratory birds from distant areas might have higher probability of harbouring exotic species of ostracods. This idea is corroborated with the presence of two alien species in our study; not only *C. novaezelandiae* but also *F. subacuta* (see Escrivà *et al.*, 2012). However, it should be noted that these ostracods might have been introduced not only by birds, but also by human activities, wind or other animals (Fox, 1965; Serramo *et al.*, 1999; Vanschoenwinkel *et al.*, 2007, 2008; Waterkeyn *et al.*, 2010; Sabagh *et al.*, 2011; Aguilar-Alberola *et al.*, 2012). Taking into account these new questions arising from our work, we suggest for future studies to increase sampling efforts on aquatic habitats of the Iberian Peninsula with high density of migratory birds and/or intense human activities, so as to carry out genetic studies of the different populations of *C. novaezelandiae* to decipher its biogeographic history, trying to detect the mechanisms and factors that have facilitated its spreading and last, but not least, find out whether or not such small organisms as alien ostracods have notable ecological effects in the colonized aquatic ecosystems.

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