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# Rice fields as facilitators of freshwater invasions in protected wetlands: the case of Ostracoda (Crustacea) in the Albufera Natural Park (E Spain)

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## Abstract

**Background:** Previous studies have identified rice fields in the Mediterranean region as 'hot spots' for the introduction of alien freshwater organisms. Consequently, special attention should be paid when rice fields are located inside or near protected areas for the conservation of native, endangered species. To analyse the relationship between rice field environmental traits and the ecology of aquatic invaders, a study of zoobenthic communities was carried out in July-September 2008 and May-August 2009 in ten rice fields located in the Albufera Natural Park (E Spain), with focus on Ostracoda.

**Results:** A total of nine ostracod species were found including four considered exotic: *Candonocypris novaezelandiae*, *Stenocypris macedonica*, *Cypris subglobosa* and *Hemicypris barbadensis*. The presence of *H. barbadensis* is remarkable, which is recorded for the first time in Europe and *S. macedonica* in the Iberian Peninsula. A checklist of Ostracoda from Albufera Natural Park is presented, including 11 exotic species, mostly unknown from Holocene sedimentary records. The analysis of ostracod assemblages showed a significant relationship between oxygen content and ostracod species associations, suggesting that their abundance are partially controlled by habitat variables in the rice fields. The exotic species *C. novaezelandiae* was more abundantly collected in samples with reduced oxygen concentrations, in contrast to other native species whose abundances were lower in such conditions.

**Conclusions:** Rice fields in Mediterranean areas present a summer inundation period which is unlikely in natural temporary water bodies in the area and which may facilitate invasion by (sub-)tropical species. Lower proportion of exotic to native ostracods is observed in less disturbed areas compared to rice fields with intense human activities. We emphasize the role of anthropogenic effects in the dispersal and colonization processes of exotic ostracods and their particular strong influence in the protected areas closest to rice fields.

**Keywords:** Alien species; Paddy fields; Ostracods; *Hemicypris barbadensis*; *Stenocypris macedonica*

## Background

Invasion by alien species is considered second only to habitat loss as a threat to global biodiversity and has its greatest impact in aquatic ecosystems (Mooney and Cleland 2001). Indeed, in recent decades, a general increase in the number of exotic aquatic invertebrates, and ostracods in particular, has been detected in freshwaters (McKenzie and Moroni 1986; Leppäkoski et al. 2002; Rossi et al. 2003).

In the Iberian Peninsula, an increasing number of freshwater ostracods are regarded as exotic species (Baltanás

1992; Baltanás et al. 1996; García-Berthou et al. 2007). Many vectors for the passive dispersal of ostracods have been recognized (Vanschoenwinkel et al. 2008; Sabagh et al. 2011; Aguilar-Alberola et al. 2012), but these do not fully explain their distribution. A vast percentage of these exotic species, mostly with subtropical and tropical origins, are established in the Mediterranean rice fields (Rossi et al. 2003; Escrivà et al. 2012). Among the studied potential vectors, the most important are probably water-birds and humans (Green and Figuerola 2005; Waterkeyn et al. 2010). In rice fields, evidences point to human activities (e.g. transport of seeds, soil and machinery moving between different areas) as the most important dispersal vector for the introduction of exotic ostracods

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from distant regions (Fox 1965; McKenzie and Moroni 1986; Rossi et al. 2003; Escrivà et al. 2012).

Inter- and intra-annual variability of environmental conditions may influence colonisation success by exotic organisms (Davis, 2009). In this framework, ecosystems with physical alteration by human activities, such as rice fields, often present alien species (Ehrenfeld 2011). Shurin (2000) demonstrated that both the probability of successful invasion by colonists and the abundance of alien species once established decline with the increasing diversity of native species. Therefore, human impacts in wetlands through rice field culturing and management activities can produce an increase in probability of alien invertebrates (and ostracods in particular) to be established in the study area.

The Albufera Natural Park (Eastern Iberian Peninsula) is mostly made up of a shallow lake surrounded by rice fields and has a large variety of native ostracods species recorded from interdune and spring ponds (Rueda et al. 2006; Rueda et al. 2013), but an impoverished community in the highly impacted lake itself (Poquet et al. 2008). In this way, this protected area can become a 'hot spot' for invasions because of the facilitation role for the entry and establishment of alien species in disturbed aquatic ecosystems. In addition, these invasion events may increase affected by climate change and globalization (Walther et al. 2009; McGeoch et al. 2010).

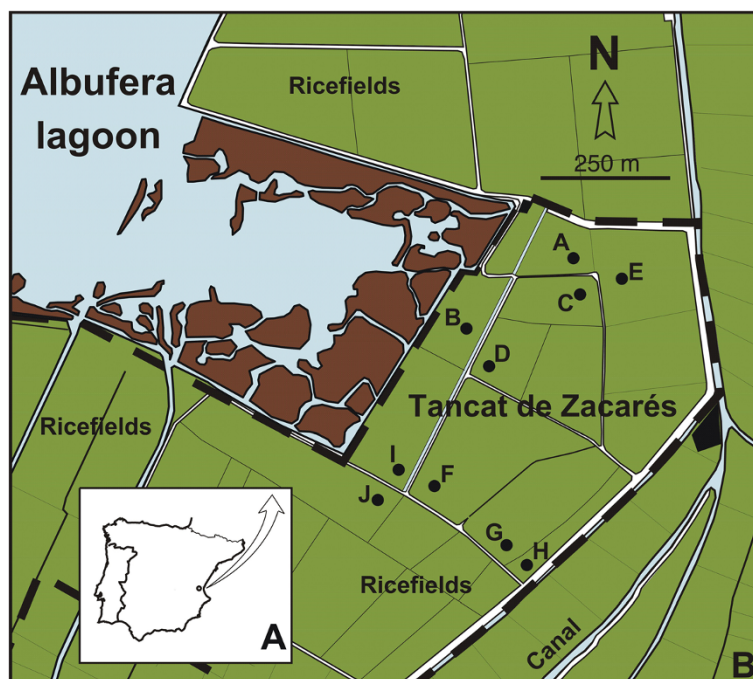
In this study, we aimed at evaluating the importance of exotic species in aquatic invertebrate communities,

exemplified by ostracods, in modified and strongly human-managed habitats such as rice fields, in comparison with less impacted environments in the same area and in contrast with paleolimnological evidence of past communities. We also planned to test whether or not environmental factors influenced significantly the structure of ostracod communities in such a highly modified environment as rice fields, subjected to strong human impacts and management.

## Methods

Rice fields studied are located in the natural preserve 'Tancat de Zacarés' (UTM: 30S 0729272/4354601), inside the Albufera Natural Park of Valencia (Eastern Iberian Peninsula), at the southern border of the lake, and covering 25.12 ha (Figure 1). Rice fields and Lake Albufera are connected by a web of irrigation canals. We sampled ten different rice fields, each named with codes 'A' to 'J'. No pesticides were used in the restricted area 'Tancat de Zacarés' during the study period.

In the field, pH, oxygen content, conductivity, salinity and water temperature were measured with portable probes. At the same time, we collected water samples for laboratory analyses of alkalinity and chloride, phosphate, nitrate, nitrite and ammonium concentrations. Benthic samples from each rice field were taken in July and September 2008 and again in May, June, July and August 2009 with a D-frame hand net with a mesh size of 100  $\mu\text{m}$  over an approximate area of 600  $\text{cm}^2$ . Sediment obtained



**Figure 1** Location of sampling sites. (A, B): (A) general location of sampling area on the Eastern Iberian Peninsula, (B) detailed location of the studied rice fields A-J in the 'Tancat de Zacarés' area in the Albufera Natural Park of Valencia.

was fixed in the field with 30% ethanol. In the laboratory, the samples were washed and sieved through a 250- $\mu\text{m}$ -mesh sieve and stored in 70% ethanol. When present, ostracods were hand-picked under a stereomicroscope and stored in 70% ethanol for specific determinations. All complete animals collected alive (i.e. belonging to the biocoenosis) were picked, so as all disarticulated valves and empty shells belonging to the taphocoenosis. Ostracod dissections were prepared following Namiotko et al. (2011), and specimens were determined using the taxonomic publications by Meisch (2000), Broodbakker (1983), Petkovski and Meisch (1996) and Karanovic (2012). We obtained SEM photos of ostracod valves with a Hitachi S-4800 microscope (Hitachi, Ltd, Chiyoda-ku, Japan) at the University of Valencia. We could not take pictures of *Dolerocypris sinensis* Sars 1903 and *Cypris subglobosa* Sowerby 1840 because of the poor preservation of their valves.

Species richness and total abundance were calculated for each sample. In addition, community differentiation was quantified with an analysis of similarities (ANOSIM) test, to compare the abundances of species between samples collected either in spring (May-June) or summer (July-September). A similarity percentage (SIMPER) analysis was used for identification and quantification of the species contributing most to the observed differences between these groups of samples.

Species-environment relationships were studied using multivariate constrained ordination. Detrended correspondence analysis (DCA) showed a wide environmental gradient ( $SD > 4$ ), indicating that some species respond unimodally to underlying ecological gradients (ter Braak and Verdonschot 1995). Consequently, canonical correspondence analysis (CCA) was performed to relate ostracod abundance to their environmental setting and the significance of habitat variables tested with Monte Carlo permutations. The DCA and CCA were performed with CANOCO 5.0 (ter Braak and Šmilauer 2012). ANOSIM,

SIMPER and Spearman correlation analyses were carried out with the software PAST 2.17 (Hammer et al. 2001).

## Results

The rice fields were flooded from April to August, and rice was sown in May. At the end of June 2008 and 2009, water was drained for 14 days. The rice was harvested in September 2008 and August 2009. In the remaining months of the rice cycle, water was drained, and the field substrate was remixed by tractors.

The rice fields included in this study were located close to each other (Figure 1) but varied notably in their environmental conditions. A summary of the physical and chemical features measured in the ten rice fields studied is shown in Table 1. Electrical conductivity at point G was, on average, higher than 3,300  $\mu\text{S}\cdot\text{cm}^{-1}$  while sites C, E and H showed values below 2,000  $\mu\text{S}\cdot\text{cm}^{-1}$ . A similar trend was observed for chloride concentration (Spearman rank correlation between conductivity and chloride concentration:  $r = 0.988$ ;  $p < 0.01$ ). High phosphate concentrations ( $>15 \text{ mg}\cdot\text{L}^{-1}$ ) were found in fields F and G, while concentrations at B, C, E and J were below the detection level ( $<0.05 \text{ mg}\cdot\text{L}^{-1}$ ). Total nitrite concentration was highest in field C (1.83  $\text{mg}\cdot\text{L}^{-1}$  on average), and values were lower than 1.0  $\text{mg}\cdot\text{L}^{-1}$  in the other points. The highest ammonia concentrations ( $>0.9 \text{ mg}\cdot\text{L}^{-1}$ ) were detected in fields F, A and I.

Nine ostracod species were recorded among the zoobenthos assemblages in the rice fields during our sampling period (Table 2). Out of these, seven were found alive in the biocoenoses (Figure 2). The most frequent species was *Heterocypris salina* (Brady 1868), followed by *Candonocypris novaezelandiae* (Baird 1843) (fields A, D, E, F and J) and *Trajancypris clavata* (Baird 1838) (fields A, D, E and F). Other species were found in lower abundance values, including *Ilyocypris gibba* (Ramdohr 1808) (fields B and D), *Hemicypris barbadensis* Broodbakker 1983 (fields B and J)

**Table 1 Mean and standard deviation values of physical and chemical variables analysed during the study period in Zacarés rice fields**

Field	N	n	Cond ( $\mu\text{S}\cdot\text{cm}^{-1}$ )	Sal ( $\text{g}\cdot\text{L}^{-1}$ )	O <sub>2</sub> (%)	WT (°C)	pH	Cl <sup>-</sup> ( $\text{mg}\cdot\text{L}^{-1}$ )	Alk/Cl <sup>-</sup>	PO <sub>4</sub> <sup>3-</sup> ( $\text{mg}\cdot\text{L}^{-1}$ )	NO <sub>3</sub> <sup>-</sup> ( $\text{mg}\cdot\text{L}^{-1}$ )	NO <sub>2</sub> <sup>-</sup> ( $\text{mg}\cdot\text{L}^{-1}$ )	NH <sub>4</sub> <sup>+</sup> ( $\text{mg}\cdot\text{L}^{-1}$ )
A	5	3	2,376 ± 34	1.2 ± 0.0	93.5 ± 29.4	25.8 ± 0.6	8.2 ± 0.7	296 ± 33	0.65 ± 0.20	0.06 ± 0.00	31.5 ± 2.8	0.12 ± 0.47	1.70 ± 0.18
B	6	4	2,906 ± 402	1.5 ± 0.2	138.2 ± 23.2	29.8 ± 2.6	8.8 ± 0.5	436 ± 70	0.38 ± 0.15	0.00 ± 0.00	33.7 ± 11.0	0.05 ± 0.04	0.58 ± 0.10
C	4	4	1,980 ± 184	1.0 ± 0.1	94.3 ± 42.1	27.8 ± 3.5	8.3 ± 0.1	209 ± 31	0.82 ± 0.26	0.00 ± 0.00	38.3 ± 12.5	0.71 ± 1.12	0.35 ± 0.13
D	4	4	2,503 ± 749	1.3 ± 0.4	112.5 ± 13.6	30.6 ± 2.3	8.7 ± 0.5	316 ± 70	0.64 ± 0.44	0.10 ± 0.20	33.7 ± 11.0	0.03 ± 0.00	0.35 ± 0.19
E	3	3	1,631 ± 218	0.8 ± 0.1	109.3 ± 50.9	29.8 ± 3.7	8.6 ± 0.2	152 ± 60	0.99 ± 0.02	0.00 ± 0.00	28.3 ± 14.1	0.33 ± 0.27	0.27 ± 1.27
F	4	3	2,610 ± 337	1.3 ± 0.2	128.6 ± 57.2	29.0 ± 1.4	8.5 ± 0.4	331 ± 61	0.48 ± 0.17	0.17 ± 0.14	25.0 ± 8.6	0.11 ± 0.12	0.97 ± 0.90
G	3	2	3,393 ± 1,034	1.8 ± 0.5	89.6 ± 33.5	29.9 ± 2.0	8.2 ± 0.2	448 ± 200	0.47 ± 0.40	0.17 ± 0.00	26.6 ± 11.5	0.19 ± 0.03	0.77 ± 0.12
H	2	1	1,845 ± 50	0.9 ± 0.0	161.0 ± 24.7	29.1 ± 0.1	8.5 ± 0.1	177 ± 3	0.73 ± 0.21	0.07 ± 0.07	30.0 ± 0.0	0.78 ± 0.18	0.05 ± 0.00
I	6	2	2,376 ± 410	1.2 ± 0.2	93.5 ± 23.5	25.8 ± 0.6	8.2 ± 0.3	296 ± 58	0.65 ± 0.19	0.06 ± 0.14	31.5 ± 12.8	0.12 ± 0.04	1.70 ± 0.29
J	4	3	2,506 ± 548	1.2 ± 0.3	83.7 ± 30.6	25.4 ± 1.0	8.3 ± 0.2	344 ± 141	0.53 ± 0.43	0.00 ± 0.00	33.0 ± 4.6	0.03 ± 0.04	0.53 ± 0.40

N, number of samples; n, number of samples with alive ostracods; O<sub>2</sub>, oxygen saturation percentage; WT, water temperature.

**Table 2 List of ostracod species found in the Albufera Natural Park**

Species	Lake Holocene	Lake (sub-) recent	Spring ponds	Rice fields	S. Llorenç pond	Interdune ponds	References
<b>Cyprididae</b> Baird 1845							
<i>Bradleystrandesia reticulata</i> (Zaddach 1844)	(1)	0 (1)					5,7
<i>Cypris</i> sp.	(1)		0 (1)				5,7
<i>C. bispinosa</i> Lucas 1849			0 (1)				7
<i>C. subglobosa</i> Sowerby 1840		0 (1)	0 (1)	0 (1)			7,11
<i>Cypridopsis vidua</i> (Müller 1776)	(3)	0 (1)	3 (1)			10 (2)	3,4,5,7,8,9,10
<i>Plesiocypridopsis newtoni</i> (Brady and Robertson 1870)						0 (1)	8
<i>Potamocypris</i> sp.	(1)						3
<i>Sarsocypridopsis</i> sp.						0 (1)	8
<i>S. aculeata</i> (Costa 1847)	(1)	0 (1)	0 (1)			16 (6)	5,7,8,10
<i>S. lanzarotensis</i> (Mallwitz 1984)			1				9
<i>Hemicypris</i> sp.	(1)						4
<i>H. barbadensis</i> Broodbakker 1983				2 (1)			11
<i>Heterocypris</i> sp.	(1)		0 (1)			5 (2)	5,7,8
<i>H. incongruens</i> (Ramdohr 1808)		0 (1)	1			2 (1)	7,8,9
<i>H. cf. rotundata</i> (Bronstein 1928)			0 (1)				7
<i>H. salina</i> (Brady 1868)	(1)	0 (1)	1(1)	9 (10)	2	25 (12)	4,6,7,8,9,10,11
<i>Dolerocypris sinensis</i> Sars 1903				0(1)			11
<i>Eucypris pigra</i> (Fisher 1851)						0 (1)	8
<i>E. virens</i> (Jurine 1820)		0 (1)				31 (5)	7,8,10
<i>Trajancypris clavata</i> (Baird 1838)				6 (4)			11
<i>Candonocypris novaezelandiae</i> (Baird 1843)				5 (2)		3(2)	10,11
<i>Herpetocypris</i> sp.	(1)						3
<i>H. cf. chevreuxi</i> (Sars 1896)	(2)	0 (1)					5,7
<i>Stenocypris macedonica</i> Petkovski and Meisch 1996				1			11
<i>S. major</i> (Brady 1868)			1 (1)				7,9
<i>Isocypris beauchampi</i> (Paris 1920)		0 (1)	2				7,9
<b>Candonidae</b> Kaufmann 1990							
<i>Candona</i> sp.	(1)						3
<i>C. angulata</i> G. W. Müller 1900	(3)	0 (1)	0 (1)				3,4,5,7
<i>C. neglecta</i> Sars 1887			0 (1)				7
<i>Fabaeformiscandona cf. brevicornis</i> (Klie 1940)			0 (1)				7
<i>F. cf. latens</i> (Klie 1940)		0 (1)				0 (1)	7,8
<i>F. subacuta</i> (Yang 1982)		0 (1)	1 (1)	2		2 (1)	2,7,8,9,10,11
<i>Pseudocandona cf. albicans</i> (Brady 1864)			1		(1)		6,9
<i>P. cf. marchica</i> (Hartwig 1889)		0 (1)	1 (1)				7,9
<i>P. pratensis</i> (Hartwig 1901)						6 (4)	8
<i>Candonopsis kingsleii</i> (Brady and Robertson 1870)			2 (1)				7,9
<i>C. scourfieldi</i> Brady 1910			1 (1)				7
<i>Cypria</i> sp.	(1)						5
<i>C. ophthalmica</i> (Jurine 1820)	(1)	0 (1)	3 (1)				3,7,9
<i>C. cf. subsalsa</i> Redeke 1936			0 (1)				7

**Table 2 List of ostracod species found in the Albufera Natural Park (Continued)**

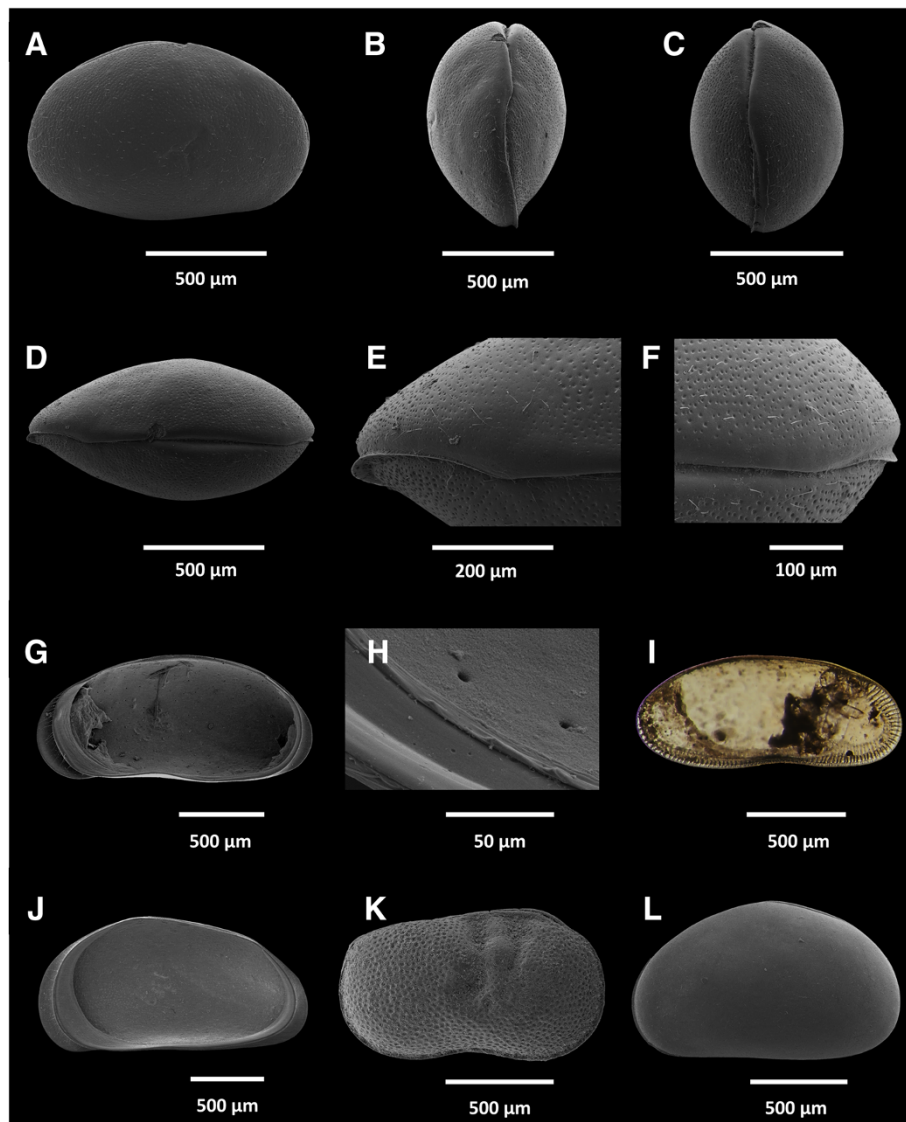
<b>Ilyocypridae</b> Kaufmann 1900						
<i>Ilyocypris gibba</i> (Ramdohr 1808)	(3)	0 (1)	4 (1)	2 (4)	5 (3)	3,4,5,7,8,9,10,11
<i>I. monstrifica</i> (Norman 1862)		0 (1)				7
<b>Darwinulidae</b> Brady and Robertson 1885						
<i>Darwinula stevensoni</i> (Brady and Robertson 1870)	(3)	0 (1)	3 (1)	2	1 (1)	3,4,5,6,7,8,9
<i>Vestalenula</i> sp.		0 (1)				7
<b>Cushmaniidae</b> Puri 1973						
<i>Pontocythere</i> cf. <i>rubra</i> Müller 1894					0 (1)	10
<i>P.</i> cf. <i>turbida</i> Müller 1894	(1)					4
<b>Cytheriidae</b> Kollmann 1960						
<i>Cyprideis torosa</i> (Jones 1850)	(3)	0 (1)	3 (1)	1	14 (13)	3,4,5,6,7,8,9,10
<b>Entocytheridae</b> Hoff 1942						
<i>Ankylocythere sinuosa</i> (Rioja 1942)					1	1
<b>Hemicytheridae</b> Puri 1953						
<i>Aurila arborescens</i> (Brady 1865)	(2)					3,4
<b>Leptocytheridae</b> Sars 1925						
<i>Leptocythere</i> sp.	(1)	0 (1)				3,7
<i>L.</i> cf. <i>porcellanea</i> (Brady, 1869)	(1)					5
<b>Limnocytheridae</b> Klie 1938						
<i>Limnocythere inopinata</i> (Baird 1843)	(2)	0 (1)	2		2 (2)	3,5,7,8,9
<i>L. stationis</i> Vavra 1891	(2)		1	0 (1)		3,5,6
<i>Paralimnocythere psammophila</i> (Flössner 1965)	(2)	0 (1)	0 (1)			3,5,7
<b>Loxoconchidae</b> Sars 1925						
<i>Loxoconcha elliptica</i> Brady 1868	(3)	0 (1)	0 (1)			3,4,5,7
<i>L. rhomboidea</i> (Fischer 1855)	(2)				0 (1)	3,4,8
<b>Paradoxostomatidae</b> Brady and Norman 1889						
<i>Cytherois</i> sp.	(1)					5
<i>C.</i> cf. <i>stephanidesi</i> Kile 1983	(1)					3
<b>Xestoleberidae</b> Sars 1866						
<i>Xestoleberis nitida</i> (Liljeborg 1853)	(3)	0 (1)				3,4,5,7
Total number of exotics/natives	<b>0/29</b>	<b>3/20</b>	<b>5/24</b>	<b>6/3</b>	<b>0/5</b>	<b>4/16</b>

Numbers of samples where alive specimens (without parentheses) or only shell remains (in parentheses) were found are indicated for each type of environment or period (sedimentary Holocene records as 'Holocene'; (sub-)recent samples for the rest). Numbers of samples for species considered (putatively) exotic are shown in italics. Numbers of exotic and native species are shown in bold. References: 1: Aguilar-Alberola et al. 2012; 2: Escrivà et al. 2012; 3: Marco-Barba 2010; 4: Marco-Barba et al. 2013a; 5: Marco-Barba et al. 2013b; 6: Mezquita et al. 2005; 7: Poquet et al. 2008; 8: Rueda et al. 2006; 9: Rueda et al. 2013; 10: Valls et al. 2013; 11: This study.

and *Stenocypris macedonica* Petkovski and Meisch 1996 collected only in field B. All of these species were also found in the taphocoenoses. One juvenile of *Fabaeformiscandona* sp. was collected but could not be further identified to species level. Furthermore, *D. sinensis* and *C. subglobosa* were only represented in the taphocoenoses (fields B and J, respectively).

The majority of the species found in this study can be considered non-indigenous species in Europe (see, e.g. Griffiths 1995; Meisch 2000). One of the most singular exotic species collected is *H. barbadensis* (Figure 2A, B, C, D, E, F), as this is the first citation of the species for Europe.

Moreover, this is the first time that *S. macedonica* (Figure 2I) is detected in the Iberian Peninsula. The other exotic species collected alive in the Zacarés rice fields are also rare in Europe. This is the case of the exotic ostracod *C. novaezelandiae* (Figure 2G, H) that has been only recorded in nearby areas (Valls et al. 2013). Pustules located in the posteroventral inner list of the left valve of *C. novaezelandiae* (Figure 2H), a trait previously recognized in individuals from a nearby population in Racó de l'Olla (Valls et al. 2013), were observed again and seem to account for a morphological trait of the species that was previously unnoticed.

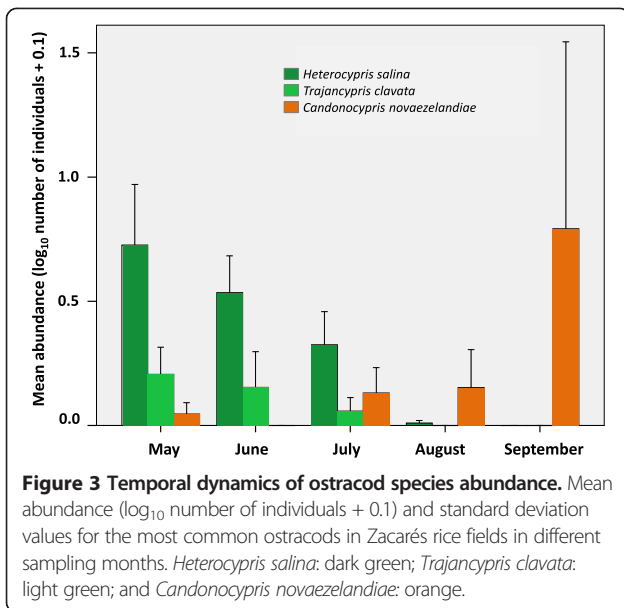


**Figure 2** SEM and stereomicroscope pictures of ostracod species found in Zacarés. (A-F) *Hemicypris barbadensis* Broodbakker 1983. (A) External view of the right valve (RV), (B) carapace in anterior view, (C) carapace in posterior view, (D) carapace in dorsal view, (E) detail of the antero-dorsal view and (F) detail of the postero-dorsal view. (G-H) *Candonocypris novaezelandiae* (Baird, 1843). (G) Inner view of the RV and (H) detail inner list of the left valve (LV). (I) *Stenocypris macedonica* Petkovski and Meisch 1996, external view of the RV. (J) *Trajancypris clavata* (Baird 1838), inner view of the RV. (K) *Ilyocypris gibba* (Ramdohr, 1808), external view of the RV. (L) *Heterocypris salina* (Brady, 1868), external view of the LV.

In order to compare the studied rice fields with other nearby habitats, we built a checklist of ostracods from the Albufera Natural Park, shown in Table 2. In all, 59 ostracod species have been recorded in the area; 27 of them were found alive and 32 were represented only in taphocoenoses. Some species present in Zacarés rice fields were never found in paleolimnologic studies carried out in Lake Albufera (Santisteban et al. 2009; Marco-Barba et al. 2013a, b). This confirms that *H. barbadensis*, *C. novaezelandiae*, *S. macedonica*, *C. subglobosa* and *D. sinensis* could be considered exotic species in this protected area. However, it should be noticed that *Hemicypris* sp. found in earlier

Holocene records could have probably colonized the area through bird dispersion from Africa, where this genus is widely distributed (Martens 1984). A similar process could be related to *Sarscypridopsis lanzarotensis* (Mallwitz 1984), common in the Canary Islands (Malmqvist et al. 1997), and perhaps in northern Africa as well. The species identified as *Stenocypris major* (Brady 1868) by Poquet et al. (2008) should be taken with caution, as these authors did not take into account the review of the genus by Petkovski and Meisch (1996).

Figure 3 shows the mean abundance of three species common to the studied Zacarés rice fields, two species



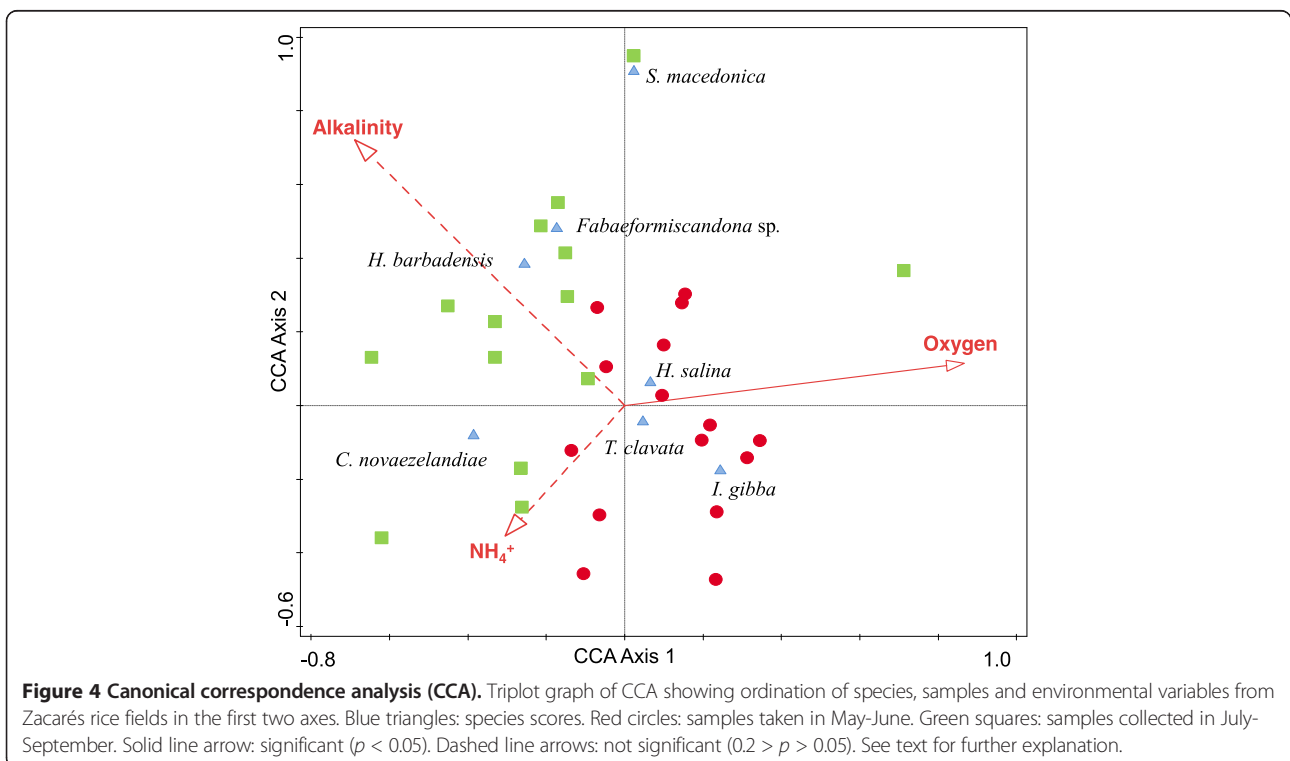
common in Mediterranean temporary habitats (*H. salina* and *T. clavata*) and one alien species (*C. novaezelandiae*). High abundance of native species and the lowest of the alien species were found in May and June. On the other hand, in July and August, abundances of native species decreased and *C. novaezelandiae* abundances increased. The results of ANOSIM indicated significant differences ( $p = 0.013$ ) in community structure between sampling periods. According to posterior SIMPER analysis, samples

collected in May-June were dominated by *H. salina* and *T. clavata*, while those collected later in summer (July-September) were dominated by *C. novaezelandiae*. These three species together accumulated *c.* 84% of the differences between these sampling periods.

CCA was carried out after applying DCA, which indicated a gradient length of 4.49 SD. In CCA, Monte Carlo permutation tests in a forward selection analysis indicated that only oxygen content was significant ( $p = 0.007$ ) in explaining ostracod community composition with the set of variables used, whereas alkalinity and  $\text{NH}_4^+$  were the next variables in order of contribution but were not significant ( $p = 0.09$  and  $p = 0.12$ , respectively) (Figure 4). Axis 1 accounted for 14.83% of the explained variance and axis 2 for 5.11%. Exotic species *C. novaezelandiae* and *H. barbadensis* were ordered in relation to low oxygen concentrations. A temporal gradient in the oxygen content was noticed: higher levels corresponded to May and June months and, by the end of summer (July-September), rice fields presented low oxygen concentration and high relative bicarbonate and ammonia concentrations.

## Discussion

Williamson (1996) proposed three main stages in biological invasion processes, i.e. arrival, establishment and spread. As Blackburn et al. (2011) pointed out, each stage can be delimited by a particular type of barrier. Understanding these barriers and factors that influence invasion success is essential to determine the role of alien species in



ecosystems. In this frame, we focused on how physical and chemical aspects and dispersal mechanisms could affect the processes of aquatic invasion by exotic Ostracoda in the study area.

In the colonization stage, physiological problems are considered the most important barrier for the establishment of alien species (Lomolino et al. 2010). In this sense, rice field ecosystems in the Mediterranean may be particularly fitted for alien species of tropical origin compared to native species (Martens and Toguebaye 1985; Forès 1988). These are temporary water bodies filled in hot, summer periods. Such combination of habitat conditions is extremely rare, if present at all, around the Mediterranean, where summers are extremely dry and temporary water bodies are filled usually in the colder months of autumn, winter or spring, but hardly ever in summer. Conversely, many tropical temporary waters experience high temperatures, and species adapted to such conditions, including ostracods such as *C. novaezelandiae*, *S. macedonica* and *H. barbadensis*, may find an empty niche in Mediterranean areas. The world distribution of these and other exotic ostracods shows that they are successful colonizers of rice fields worldwide, but particularly in (sub-)tropical areas. *H. barbadensis* was reported in the Barbados and Japanese rice fields (Broodbakker 1983; Okubo 1990) and from a permanent lake in Jamaica (Holmes 1997). *Candonocypris novaezelandiae* has indeed a wider distribution area, as it is present in lakes and ponds in Australia, New Zealand, New Caledonia islands, China, Spain and South Africa (Sars 1924; De Deckker 1981; De Deckker 1983; Martens 1984; Eagar 1994; Martens et al. 1998; Yu et al. 2010; Valls et al. 2013), in the river Nile (Ebtessam 2010) and in fishponds in Japan (Okubo 1975). Also, *C. subglobosa* has been commonly cited in worldwide tropical wetlands, usually including rice fields, from Africa, America, Asia and Europe (Martens and Toguebaye 1985). These authors remark on the tropical-like functioning of rice field ecosystems in temperate areas.

In spite of this facilitation to exotic ostracod species to colonize the Albufera Natural Park, particularly in rice fields and surroundings (Table 2), most of these are not usually found outside this type of environments. However, some exotic species seem to be pre-adapted to tolerate the conditions of other temperate water bodies. This might be the case for *C. novaezelandiae*, which is not only found in paddy fields in the newly colonized continent (e.g. Valls et al. 2013) but is also able to jump to other types of (disturbed) water bodies such as reservoirs (Escrivà et al. 2014). A similar case is exemplified by *Fabaeformiscandona subacuta* (Yang 1982), an exotic ostracod of Asian origin in the Iberian Peninsula, which apparently dispersed from rice fields to the Albufera Natural Park and other inland areas (Escrivà et al. 2012). This species is widely distributed, and its invasion history reflects the

potential problem that we discuss in the present survey. In fact, the presence of *F. subacuta* in Iberian reservoirs could be related to low oxygen concentration (Escrivà et al. 2014), similarly as to the relationship found in this survey of rice fields where the exotic *H. barbadensis* and *C. novaezelandiae* related to the more reduced environments, with low oxygen content in the negative side of the CCA ordination axis (Figure 4). *C. novaezelandiae* and *H. barbadensis* were found only in summer (July and August) (Figure 3) when oxygen presented low values (Figure 4), but alkalinity and ammonium increased. Hence, these conditions where exotic species were present correspond to highly eutrophicated and disturbed environments, with strong dominance of respiration processes related to organic matter decomposition. However, these findings still need corroboration because oxygen content can be highly variable in rice fields and other aquatic systems (Quesada et al. 1997), and we did not measure daily variability to ascertain that our measures taking during the day correlated with night oxygen content values (expected to be much lower). Anyway, under some circumstances in which water bodies are closer to their pristine state, the high diversity of native ostracods might hinder the invasion by exotic species, i.e. the diversity-invasibility hypothesis (but see Davis 2009). The relatively high ostracod diversity in this natural park (Table 2) could therefore reduce the possibility of further colonization by alien species, which seem to be more successful in the disturbed rice field areas.

Other studies about ostracod diversity in rice fields in Japan (Okubo 1990), Italy (Fox 1965; Rossi et al. 2003) and Spain (Forès 1988) also show high levels of xenodiversity. This suggests that dispersal and colonization abilities of exotic ostracods in dynamic and anthropized environments could be higher than those in more preserved habitat types. *F. subacuta* was most probably transported to the Iberian Peninsula by human activities. Rice trading might have been the vector accounting for such long-distance ostracod dispersal, as discussed by McKenzie and Moroni (1986) and others (Fox 1965; Forès 1988; Rossi et al. 2003). Devising measures to prevent these dispersal events by human activities is therefore imperative (McGeoch et al. 2010).

## Conclusions

The ostracod assemblages analysed in Zacarés rice fields reveal a significant relationship between species composition and environmental conditions, in particular reduced oxygen content in summer compared to spring samples, suggesting species sorting effects in the structuring processes of these communities in the area. Among the species found, the high proportion of exotic species of (sub-)tropical origin is remarkable, including *H. barbadensis*, recorded for the first time in Europe, *S. macedonica* and *C. novaezelandiae*. A higher ratio of native to exotic



species is found in rice fields compared to other environments of the Albufera Natural Park, suggesting that these habitats function as facilitators for the establishment of alien species. Rice fields are probably the only temporary environments that follow the opposite trend to most other temporary Mediterranean water bodies, being flooded during the warmest and driest summer months. This human-managed artificial hydroperiod allows the colonization of the area by exotic ostracods from other continents, some of which might invade other habitat types, with unknown consequences for the native fauna.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

JR and FM conceived the research and performed the analysis. JR collected the specimens and field data. JR and LV performed the laboratory work. LV wrote the first version of the manuscript with help from JR with the figures. JR and FM made modifications to the manuscript. All authors read and approved the final manuscript.

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