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PRIMARY RESEARCH PAPER

Dynamics of sexual and parthenogenetic populations of *Eucypris virens* (Crustacea: Ostracoda) in three temporary ponds

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Abstract *Eucypris virens* is a freshwater ostracod in which both sexual reproduction and parthenogenesis occur. Sympatric coexistence of both reproductive modes is known in zones of overlap.

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This renders the species a potentially valuable model organism to study the ‘queen of evolutionary problems’, i.e. why sex is so successful despite its costs (paradox of sex). In order to maximally exploit this potential, a broad knowledge of the species’ ecology is essential, including an understanding of its life history and population dynamics. Here, the phenology of the species was followed in three temporary ponds through monthly (Spain) or fortnightly (Poland) samplings, throughout an inundation period. This study confirms the wide ecological tolerances of *E. virens*. Although the species is generally assumed to be univoltine, two hatching periods were observed in the Spanish sites. Biotic interactions, especially predation, appear to be the important determinants of population dynamics in long-hydroperiod sites. Abiotic conditions may influence population dynamics through their impact on egg hatching. In the site with male presence, the initially female-biased sex ratio evolved towards a balanced sex ratio through the season. No consistent differences in limb morphology were observed between females originating from the three study sites. On the other hand, valve size of adult females varied among sites, possibly influenced by local environmental conditions (mainly salinity and pH) as well as the expected genetic diversity.

Keywords Ostracoda · Phenology ·
Temporary ponds · Ecological preferences

Introduction

Ostracods are small bivalve crustaceans abundant in nearly all freshwater and marine habitats, but found also in interstitial and semi-terrestrial environments (Horne et al., 2002). The group exhibits a high taxonomic diversity (ca. 10000–15000 extant species; Kempf, 1980–2006, 1986–2008), an extensive fossil record (Horne & Martens, 1998; Martens et al., 2008) and an extraordinary diversity of life history strategies (Chaplin et al., 1994; Butlin et al., 1998; Geiger, 1998). This in combination with their relatively short generation time and the high frequency of transitions from sexuality to asexuality observed in certain genera (Chaplin et al., 1994; Butlin et al., 1998) denotes the Ostracoda as valuable model organisms to study the fundamental evolutionary questions.

Reproduction in freshwater ostracods displays a variety of forms: the ‘ancient asexuals’ with unknown sexual relatives, genera and/or species with co-occurring parthenogenetic lineages (asexuals) and sexual lineages (sexuals) and obligatory sexual populations (Chaplin et al., 1994; Judson & Normark, 1996; Butlin et al., 1998). Evolutionary questions have so far been tackled mainly by studying the (palaeo)geography and the phylogeny of ostracods with different modes of reproduction (Griffiths & Horne, 1998; Horne et al., 1998; Schön et al., 2000). Chaplin et al. (1994) found that asexual ostracod taxa are relatively more successful in freshwater habitats of recent origin, whereas sexuals are commonly found in marine environments and post-glacial lakes. This points to the supreme colonization potential of asexuals and to their vulnerability to local displacement by sexuals in the absence of environmental change (Bell, 1982).

Theory predicts a twofold advantage for asexual populations compared to their sexual relatives, because the latter suffer from the cost of male production (Maynard Smith, 1978). Still, sexual populations persist and compete directly with their asexual relatives, at times even within the same pond (e.g. Schön et al., 2000). A straightforward approach to study the ecological and evolutionary significance of sex is to compare life history traits and population dynamics between sexuals and asexuals. Geiger (1998) conducted a comparative analysis involving life history traits of sexual and asexual freshwater ostracod species. His analysis suggested that species-

specific adaptations, such as the capacity to produce resting eggs, rather than features associated with the mode of reproduction determine the population dynamics of freshwater ostracods.

We monitored population dynamics in three temporary ponds of sexual and/or asexual lineages of the freshwater ostracod *Eucypris virens* (Jurine 1820). High clonal diversity within sites and regions (Schön et al., 2000; Rossi et al., 2008) and substantial morphological variability (Tétart, 1982; Martens et al., 1992; Baltanás et al., 2000; Baltanás, 2002) have been reported in *E. virens*. Recent molecular analyses show evidence of multiple origins of asexuality from genetically distant sexual lineages. These sexual populations are characterized by deep phylogenetically divergence, enough to be considered as a cryptic species complex (Bode et al., in press). Most ponds have been colonized repeatedly, as assessed by the presence of multiple more or less related parthenogenetic lineages within one pond (Adolfsson et al., submitted). Prior to these findings, *E. virens* was regarded as a single species on the basis of morphology. Laboratory studies suggest that coexistence of *E. virens* lineages may be enhanced by differences in hatching phenologies and developmental rates (Otero et al., 1998; Martins et al., 2008), but so far field evidence is lacking. We followed the dynamics of two parthenogenetic populations and one bisexual population (coexistence of sexually and asexually reproducing lineages) throughout one inundation period. The importance of environment and mode of reproduction for the short-term dynamics of these *E. virens* populations was assessed in a qualitative way by linking hatching patterns and changes in the population structure and density with variations observed in the abiotic and biotic environments.

Materials and methods

Study species

Eucypris virens is a freshwater ostracod species complex, traditionally regarded as a morphospecies. Parthenogenetic populations are present throughout Europe, North Africa, North America and Asia (Baltanás, 1994; Meisch, 2000; Semenova, 2005) and are also known from Australia (Radke et al., 2003), New Zealand (Barclay, 1968; Eagar, 1971)

and some Sub-Antarctic islands (Tétart, 1982; Pugh et al., 2002). Males are recorded from the circum-Mediterranean area and central Asia only (Meisch, 2000). The species is common in temporary ponds, but has been recorded in a wider variety of habitats (Meisch, 2000), with salinity ranges from 0 to 5‰ (Hiller, 1972). It is a thermoeuryplastic species (Nüchterlein, 1969) with tolerance for eutrophic waters (Mezquita et al., 2001; Gifre et al., 2002). The few published population density records indicate relatively low population densities (compared to other ostracod species) ranging from 13 to 200 ind. m⁻² (Baltanás, 1994). The growing and reproductive periods are winter and spring (Alm, 1915; Baltanás, 1994; Mezquita et al., 1999) but more eurychronic populations, present throughout the year, may also occur (Altınışli, 2001). Although the species is typically regarded as univoltine, a second hatching period is possible (Meisch, 2000). Hatching rates are highly variable among populations and lineages, and depend on the prevailing environmental conditions (Otero et al., 1998; Vandekerckhove et al., 2007; Martins et al., 2008). Adult sexual and asexual *E. virens* can lay two types of eggs: subitaneous (immediate hatching) and diapausing eggs (desiccation-resistant resting eggs). The latter allow the persistence of populations in periods of drought or freezing and are important passive dispersal units (Horne & Martens, 1999). *E. virens* may also survive short drought periods embedded in the mud (O. Schmit, unpublished data). Post-embryonic development includes eight juvenile stages prior to the final moult within 4–5 weeks (Sweden: Alm, 1915) to 6–7 weeks (Germany: Hiller, 1972), depending on temperature and other physical and chemical conditions.

Field work

Sampling was carried out in three strategically selected temporary ponds. The selection contains one pond in Northern Europe [pond Jabłoniowa (JAB), Gdańsk, Poland: N 54°19'54", E 18°33'51", 91 m asl] where only asexual *E. virens* populations are found and two ponds in the Mediterranean region (Spain). Within the Mediterranean, one pond was sampled in an area where *E. virens* males are absent [pond Malladas 3 (MA3), València: N 39°19'27", W 0°18'18", 0 m asl]. The second pond was sampled in

a region where males had been previously recorded [pond Monfragüe 4 (MF4), Extremadura, Spain: N 39°54'39", W 6°03'48", 432 m asl]. As such, the selection of ponds allowed us to uncouple the effects of climate/latitude and reproductive mode. The Spanish sites are isolated ponds, located in a natural park and along cattle pathways, respectively. The Polish site JAB is composed of a temporary pond connected to a few disused ditches in a former agricultural land bordering a heavily used road on the outskirts of Gdańsk city limits.

Sampling was carried out from the first (ice free) water presence until the site was recorded as dry (at the end of the rainy season). Ponds were visited monthly (Spain) or fortnightly (Poland), at approximately the same time of the day (late morning), to avoid major effects of environmental daily variations. Ostracod and macroinvertebrate communities were sampled semi-quantitatively by sweeping the bottom (superficial sediment and adjacent water column) of the pond using a hand net (width: 25 cm; mesh size: 120 µm). The sampling was intended to characterize each pond as a whole; therefore, the procedure was carried out in four randomly chosen locations and combined to obtain a sampled surface area of ca. 1 m² (sampling unit, coded as s.u.). Biotic samples were preserved in 70% ethanol.

At each sampling occasion, water temperature, pH, conductivity and oxygen content were measured in situ using electronic probes. Water samples were collected with a 3-l beaker at different depths and locations. Sediment samples were obtained using a Plexiglas corer (5 cm diameter). Only the upper 5 cm of sediment was retained, and samples taken at five different locations were combined in a plastic bag. Sediment and water samples were transported in a cool box and stored at 4 or –20°C, respectively, upon arrival in the laboratory.

A detailed description was made of the pond morphometry and visual habitat and environmental characteristics (e.g. land use, macrophyte composition and coverage). Climate characteristics were assessed based on regional daily measurements of precipitation, radiation and air temperature (Spain: Meteorological stations in Valdeñigos for MF4 and Sagunt for MA3; Poland: Meteorological stations in Gdańsk and Gdynia). Variables were calculated as monthly (Spain) or fortnightly (Poland) averages, in correspondence with the sampling frequency.

Laboratory work

Ostracods and macroinvertebrates were sorted using a stereomicroscope (40× magnification) and identified under a stereomicroscope (100× magnification) or standard light microscope (1000× magnification) using the keys of Meisch (2000) and Tachet et al. (2000). All adult ostracods were counted and identified to the species level when possible. In juvenile *E. virens*, the instar stage was determined according to Smith & Martens (2000); the intact left valves were measured in two dimensions using a stereomicroscope and the software image analysis system Lucia (Laboratory Imaging, Prague; 50× magnification; 0.01 mm accuracy). When ostracod densities in a given sample exceeded 1000 ind. s.u.⁻¹, the identifications and measurements were limited to the animals found on a randomly selected fraction of the total sample (usually 1/2). Macroinvertebrate categorical densities were estimated as (1) 1 ind. s.u.⁻¹, (2) 2–10 ind. s.u.⁻¹, (3) 11–100 ind. s.u.⁻¹, (4) 101–1000 ind. s.u.⁻¹, (5) >1000 ind. s.u.⁻¹.

Major anion (alkalinity, chloride and sulphate) and nutrient (nitrate, ammonium and phosphate) concentrations were quantified using standard analytical methods (APHA-AWWA-WEF, 1992). Chlorophyll *a* concentration was quantified spectrophotometrically after extraction of the pigments of algae retained by Whatman GF/F filters in acetone-dimethylsulfoxide (Shoaf & Liem, 1976), applying the equations given by Jeffrey & Humphreys (1975). Major cation concentrations (Ca²⁺, K⁺, Mg²⁺, Na⁺ and Sr²⁺) were measured by inductively coupled plasma mass spectrometry (ICP-MS). Density, porosity and organic matter content of the sediment samples were determined by weight measurements before and after heating in an oven (50°C) and a muffle furnace (450°C) (APHA-AWWA-WEF, 1992).

Statistical analyses

In order to test for differences among sites in valve dimensions, only measurements on adult females were used. This is because males and females are known to differ in size (Meisch, 2000), and juvenile instars cannot be sexed consistently. Length and height were compared among sites with univariate analyses of variance (ANOVA). Tukey's post hoc tests were used for pairwise comparisons.

Assumptions of normality and homogeneity of variances were tested using the Kolmogorov–Smirnov and Levene statistic tests, respectively. Analyses were calculated using SPSS_{win} 15 (SPSS Inc., 2006).

Principal component analysis (PCA) was applied to summarize the most important environmental gradients in our sites to compare these with observed population structures. Prior, detrended correspondence analysis (DCA) was applied to assess the range of the data set used before ordination analyses. Our data presented narrow ranges (SD < 2), and linear ordination models were applied (Lepš & Šmilauer, 2003). From a total of 22 variables (Table 1), 14 variables were used for the PCA after eliminating strongly correlated variables (R > 0.8, Spearman's correlation coefficients on log transformed values). Environmental variables were centred and divided by standard deviation (Lepš & Šmilauer, 2003). Ordination analyses were performed with Canoco_{win} 4.5 (Ter Braak & Šmilauer, 2002); normality assumptions and Spearman's correlation tests were analysed with the statistical program R_{win} 2.8.1 (R Development Core Team, 2008).

Results

Pond characteristics

JAB, MA3 and MF4 are small and shallow ponds (max. surface area: 4300, 2500 and 800 m²; max. depth: 0.36, 0.14 and 1.5 m; respectively). MA3 has a sandy substrate overgrown mainly with grassweed. Algae and periphyton were observed at the end of the season. MF4 has a clay substrate; macrophyte coverage was low, increasing by the end of the season (grass, *Ranunculus* sp.); algae and periphyton were abundant throughout the season. JAB has a loamy substrate, with constant high macrophyte coverage (grass and reeds), while algae and periphyton were present in the last two sampling occasions only.

In Spain, the rainy season began in September/October 2005. In the shallow site MA3, the last monthly sample was collected in March 2006 with the site recorded dry in December for several weeks. Site MF4 was inundated until May 2006. In the Polish site JAB, sampling started in April 2006 after the melting of the ice. This pond was visited fortnightly, until it dried out mid-June 2006, to

Table 1 Mean, minimum and maximum values for environmental variables for the three sampled ponds

Variable (unit)	Code	Mean			Minimum			Maximum		
		JAB	MA3	MF4	JAB	MA3	MF4	JAB	MA3	MF4
Mean air temperature (°C) ^a	Air temp.	9.2	13.5	9.7	5.0	9.7	5.0	12.9	19.4	15.5
Precipitation (mm m ⁻²) ^a	Precip.	2.6	1.2	2.6	0.3	0.1	1.1	6.2	2.7	7.9
Radiation (MJ m ⁻²)	Radiat.	15.0	12.3	10.9	11.5	12.1	6.7	21.8	12.5	19.8
Conductivity (µS cm ⁻¹) ^a	Cond.	919	1890	51	682	723	39	1195	2830	63
pH ^a	pH	7.12	8.17	7.31	7.01	7.89	6.54	7.34	8.40	8.56
Dissolved oxygen (mg l ⁻¹) ^a	O ₂	8.1	8.1	6.9	6.0	3.1	0.3	10.8	12.8	10.6
Water temperature (°C) ^a	Temp.	16.2	14.8	10.7	13.9	7.7	5.2	21.5	24.5	14.9
Alkalinity (mEq l ⁻¹) ^a	ALK	2.2	6.6	0.3	1.6	2.4	0.2	2.6	9.3	0.5
Sulphate (mg l ⁻¹)	SO ₄	24	130	16	5	38	10	42	250	31
Chloride (mEq l ⁻¹)	Cl	5.5	10.5	0.2	4.0	4.0	0.1	7.2	16.2	0.3
Calcium (mg l ⁻¹)	Ca	69	85	4	55	25	3	78	146	4
Magnesium (mg l ⁻¹) ^a	Mg	8	80	2	5	25	1	9	129	3
Sodium (mg l ⁻¹)	Na	101	234	4	65	88	3	154	373	5
Potassium (mg l ⁻¹)	K	13	17	3	10	8	1	15	27	7
Strontium (mg l ⁻¹)	Sr	0.19	0.67	0.02	0.12	0.20	0	0.22	1.06	0.04
Nitrate (µmol l ⁻¹) ^a	NO ₃	33	48	46	12	36	35	46	69	62
Ammonium (µmol l ⁻¹) ^a	NH ₄	5	50	5	2	5	3	8	169	15
Phosphate (µmol l ⁻¹) ^a	PO ₄	2.9	0.7	1.2	0.6	0.1	0.5	5.7	1.4	2.4
Chlorophyll <i>a</i> (µg l ⁻¹) ^a	Chl <i>a</i>	24.8	1.9	8.0	5.0	0.3	1.8	48.8	6.1	19.7
Organic matter sediment (%) ^a	%OM	6.7	2.9	5.4	4.9	1.8	4.2	10.6	3.9	9.0
Open water surface (%) ^a	Open	2	53	78	0	10	60	10	95	98
Emerged macrophyte coverage (%)	Emerg.	51	63	6	15	5	0	90	90	20
Submerged macrophyte coverage (%)	Subm.	47	2	10	10	0	0	85	10	30

^a The variable was included in the principal components analysis (see Fig. 1)

obtain a comparable number of samples per pond (MA3 and JAB: $n = 5$, MF4: $n = 7$).

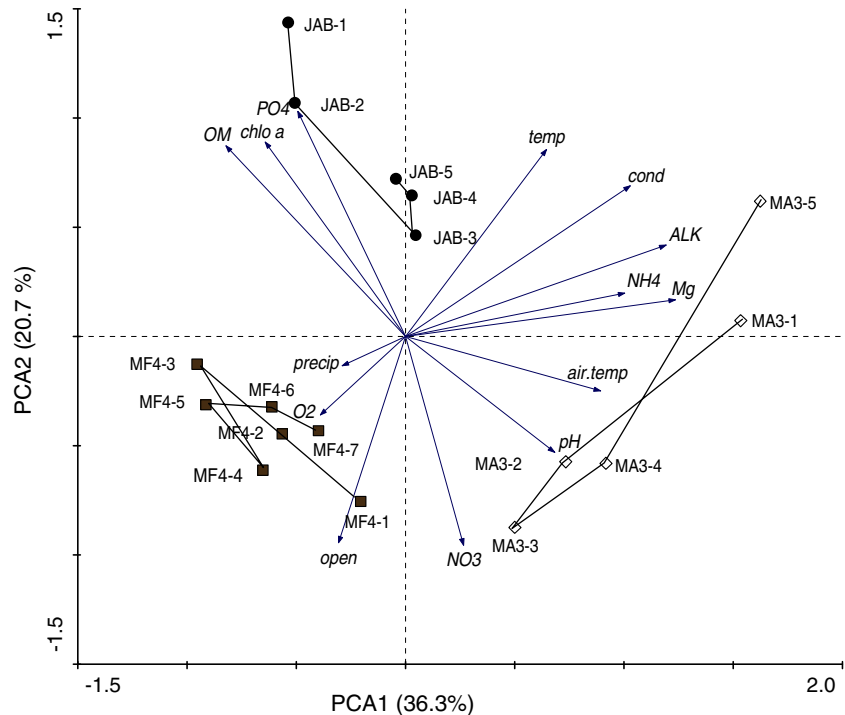
The main environmental gradients, both within and between sites, were identified by PCA. The first two dimensions of the PCA accounted for 57.0% of the total observed environmental variance. PCA 1 (36.3%) is positively correlated with ionic concentration indicators (Mg²⁺ concentration, alkalinity having the highest correlation score) and NH₄⁺ concentration. This axis separates site MA3, with positive axis scores, from sites JAB and MF4 (Fig. 1). The second dimension (20.7%) provides a division between sites JAB and MF4 according mainly to their trophic level: JAB has high PO₄³⁻ and chlorophyll *a* concentrations and high organic matter content, while MF4 has reduced values of these variables and low ionic concentration. No environment changed over time in a directional way

in the PCA biplot area. Seasonal variation in the environment was most pronounced in MA3 along both PCA axes.

A total of 16 ostracod species were identified in the three sites (Table 2). In site JAB, both the highest species richness (11 species) and the highest total density (> 80.000 ind. s.u.⁻¹) were observed. MA3 had the lowest ostracod species richness (four species), with high dominance of two ecologically tolerant species: *E. virens* and *Heterocypris incongruens*. In MF4, *E. virens*, *Candona neglecta* and *Ilyocypris gibba* were most abundant in October, while *Herpetocypris chevreauxi*, a species common in longer hydroperiod water bodies, dominated towards the end of the sampling season.

Total macroinvertebrate taxon richness (excluding Ostracoda) was high in MF4 and JAB (28 and 22 taxa, resp.) and low in MA3 (10 taxa) (Table 2;

Fig. 1 PCA ordination diagram based on 14 environmental variables measured at 17 occasions in three study sites (JAB, MA3 and MF4). Environmental variables are listed and coded in Table 1. The number after the pond code indicates the sampling visit (see Table 2 for dates)



complete list available in Supplementary Material—Appendix). In MF4, macroinvertebrate diversity increased as submerged and emergent macrophytes became more abundant. No clear trend in taxon richness was observed in MA3 and JAB, where macrophyte coverage remained more constant throughout the season. The density of competitors (detritivores and herbivores) was continuously high in JAB and increased gradually together with the density of predators in MF4. Conversely, large branchiopods were initially abundant in MF4, but disappeared towards the end of the season.

Valve size of *Eucypris virens*

Valve dimensions of adult females differed among sites (ANOVA: length: $df = 2$, $F = 72.4$, $P < 0.001$; height: $df = 2$, $F = 54.3$, $P < 0.001$). MA3 adult females were on average smaller (length: 1.65 ± 0.15 mm; height: 1.03 ± 0.09 mm) than females from JAB and MF4 (length: 1.97 ± 0.06 mm and 1.93 ± 0.16 mm; height: 1.21 ± 0.03 mm and 1.17 ± 0.1 mm, resp.). Figure 2 shows the size for all instar stages in the three sites. In stages A-1 and A-2, a similar pattern as described in the adult stage could be observed (both

for length and height), despite possible inclusion of males. For the younger development stages, a large overlap in size between consecutive instars was observed for each site. *E. virens* adult females collected in MA3 and MF4 were more variable in size compared to adult females collected in JAB (e.g. range in length, MA3: 1.56–1.88 mm, MF4: 1.86–2.17 mm, JAB: 1.82–2.05 mm). This variability diminishes in younger stages but could also result from a smaller sample size.

No substantial differences were observed in the limb morphology between females originating from the three study sites. However, some minor discrepancies in the chaetotaxic pattern during progressive developmental appearance of the limbs were encountered compared to the pattern described by Smith & Martens (2000). Nevertheless, these incongruities were not constant within the sample.

Eucypris virens: population dynamics

Eucypris virens was recorded in the first sample for all the three sites (Table 2). In all the sites, maximum *E. virens* densities exceeded $100 \text{ ind. s.u.}^{-1}$ and

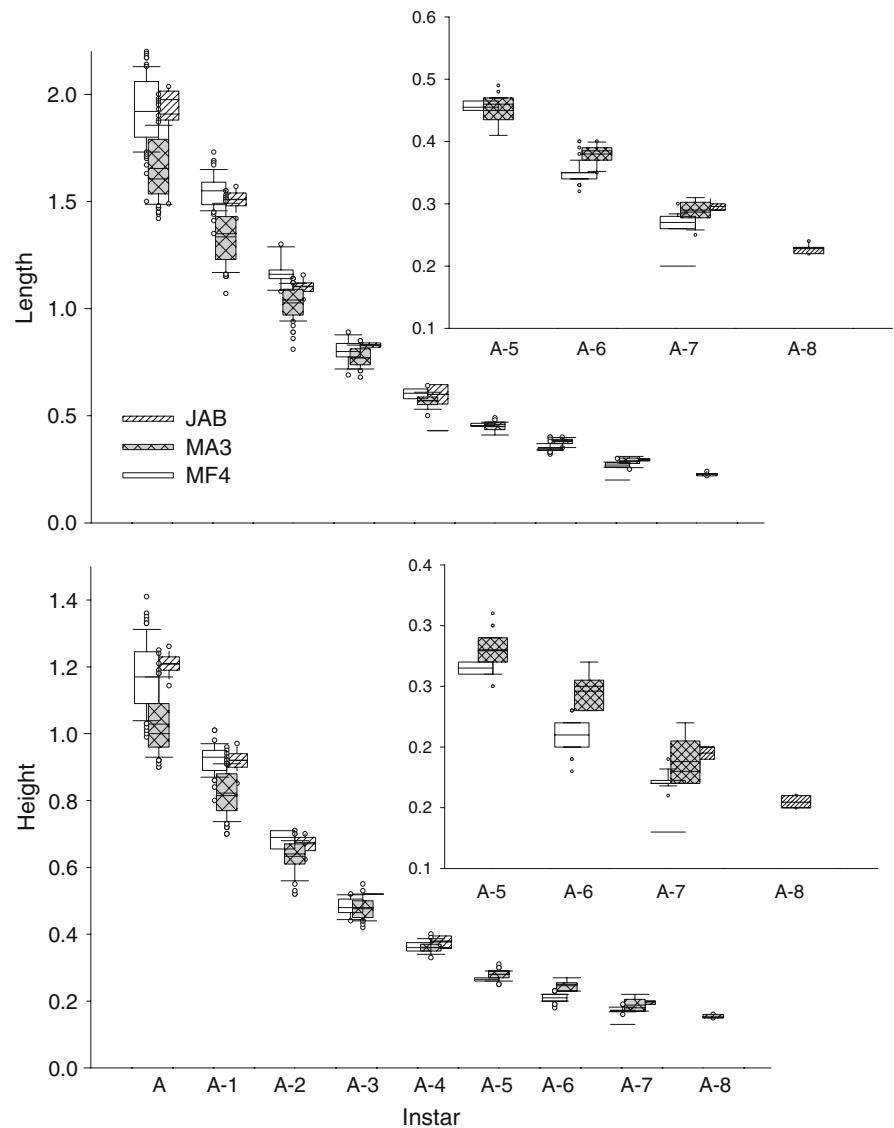
Table 2 Categorized densities of ostracod species and macroinvertebrate taxa

Species/taxon	JAB					MA3					MF4						
	3/4/ 2006	15	31	45	63	19/10/ 2005	41	85	117	146	21/10/ 2005	20	44	76	112	143	181
<i>Bradleystrandesia reticulata</i> (Zaddach, 1844)		3															
<i>Candona neglecta</i> Sars, 1887										3	2	2					
<i>Cyclocypris</i> spp.				5	5												
<i>C. laevis</i> (Müller, 1776)	5	5	5	5	5												
<i>C. ovum</i> (Jurine, 1820)	4	5	4	4	4												
<i>Cypria ophthalmica</i> (Jurine, 1820)	4	5	4	4	5												
<i>Cypridopsis vidua</i> (Müller 1776)				5	5												
<i>Cypris pubera</i> (Müller, 1776)	5	5	5	4	4												
<i>Eucypris virens</i> (Jurine, 1820)	4		2	4		3	4	3	4	4	4	4	3	3	2	3	
<i>Herpetocypris chevreuxi</i> (Sars 1896)													2			2	3
<i>Heterocypris incongruens</i> (Ramdohr, 1808)						4	3	3	2	4	1	1					
<i>Ilyocypris gibba</i> (Ramdohr, 1808)										4	2		2	1			
<i>Notodromas monacha</i> (Müller, 1776)					3												
<i>Paralimnocythere psammophila</i> (Flössner, 1965)										1							
<i>Pseudocandona compressa</i> ^a (Koch, 1838)	5	5	5	4	5	1		1									
<i>Sarscypridopsis aculeata</i> (Costa, 1847)										2							
<i>Tonnacypris lutaria</i> (Koch, 1838)		1															
Macroinvertebrates																	
Arachnida Hydracarina	3	3	3	3	2	2		2	3		2	1					2
Branchiopoda (-Cladocera)										4	3	2	2	1	1		
Cladocera		5	5	5	3		3	4	4		2	2	2	2	2	2	4
Coleoptera	2	2							2								
Collembola	2	3							2								
Copepoda	4	5	4	4							3	2	3	3	2	2	
Diptera	3	4	4	4	4		2	3	4		2	2	1	1	3	4	
Ephemeroptera									2		1				2	2	
Gastropoda	2	3								2							
Heteroptera										2			2	1	2	3	
Hirudinea				1													
Nematoda				3						2	2	2	3	2	2	3	
Odonata	1																2
Oligochaeta	2	1					1					1	1		2	2	
Platyhelminthes											3	2	3	2	3	4	
Trichoptera	3	2	3	2													

The date indicated is the day of the first sampling occasion per site, i.e. day 0; subsequent numbers refer to the number of days passed between the first sampling and the day of sampling. Scale: (1) 1 ind. s.u.⁻¹, (2) 2–10 ind. s.u.⁻¹, (3) 11–100 ind. s.u.⁻¹, (4) 101–1000 ind. s.u.⁻¹, (5) >1000 ind. s.u.⁻¹

^a *Pseudocandona* sp. for MA3

Fig. 2 Box plots of length and height (mm) for the nine life stages (A—adult to A-8—first instar) of *E. virens* for the three sampling sites



strong density fluctuations occurred within the season. The highest *E. virens* density was observed in JAB (ca. 400 ind. s.u.⁻¹, 3rd April 2006). Still, *E. virens* was not numerically dominant in JAB, as *Pseudocandona compressa* and *Cyclocypris* spp. were several orders of magnitude more abundant. In MA3, *E. virens* co-dominated the ostracod community together with *H. incongruens*, while in MF4 the species was usually the dominant ostracod species. *E. virens* was present until complete desiccation of the site in MA3. In site JAB, *E. virens* was not recorded in mid-April and June. In MF4, the species was not recorded after March, while water was present until May.

In all the sites, a massive hatching event was observed within 1 month after inundation/thawing. The distribution of instars over time suggests that hatching from resting eggs continued for several weeks (Fig. 3). In MA3, a second hatching peak occurred after a short period of desiccation in the middle of the wet season (December 2005). Although the magnitudes of both hatching peaks were comparable, the second gave rise to a much higher concentration of adults, which exceeded many times the density of hatchlings. An increase in the concentration of adults at the end of the second inundation event coincided with a dramatic decrease in pond surface (from 24 to 7 m²). In MF4, a second small

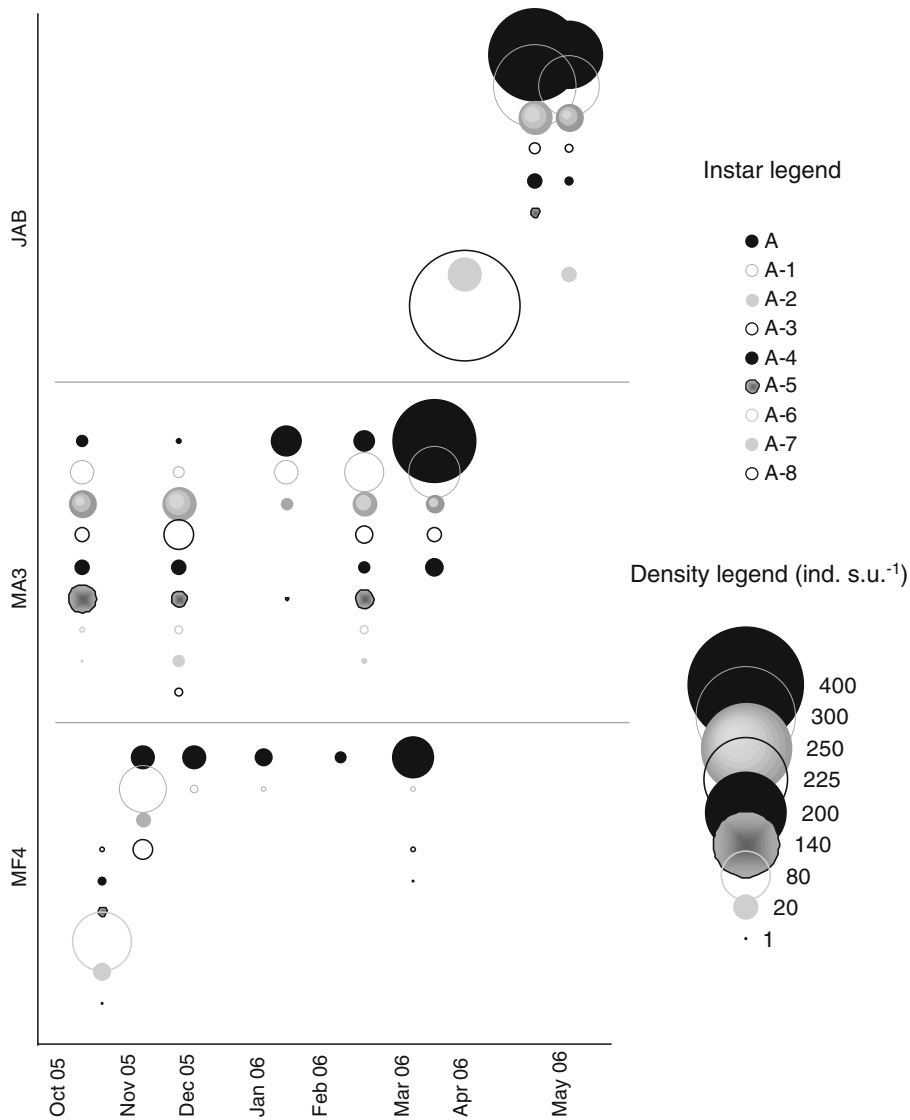


Fig. 3 Temporal change in the density of *E. virens* for the nine life stages (in the order: A—adult to A-8—first stage) in the three study sites. The circle size is proportional to the density of the different life stages (ind. s.u.⁻¹)

cohort entered the population ca. 5 months after the initial filling of the pond. Here, the changes in pond volume were much less pronounced, and densities were gradually decreasing until the second cohort entered the population. In May, *H. chevreuxi* was the only ostracod species recorded. The initially female-biased sex ratio in MF4 evolved towards a balanced sex ratio before *E. virens* disappeared (male:female sex ratios at visits 2 to 6: 1:4.5; 1:1.9; 1:2.3; 1:1.3; 1:1.0). The inundation period of JAB was much shorter, and only one hatching event was observed.

E. virens was not recorded in two of the five samplings.

Discussion

Eucypris virens: valve dimensions

Valves are important diagnostic elements for the identification of ostracods. Nevertheless, their shape and size are known to covary with ionic conditions,

conductivity and pH (e.g. Alcorlo et al., 1999). In our study, we verified that adults originating from site MA3, with the highest salinity values and ionic concentrations, were smaller than those of the other sites. This is in agreement with an observation by Mezquita et al. (2000), who found that the size of *Cyprideis torosa* (Jones, 1850) individuals correlated negatively with the salinity level of the pond, but there might be an influence of other factors such as temperature or photoperiod (Alcorlo et al., 1999). We also observed higher variation in valve dimensions in the Spanish sites. It should be noted that for JAB, only females from a single sample were measured, while for MA3 and MF4 females from several samples were measured, and therefore, a reduced variability is expected for JAB. Notwithstanding possible environmental effects on shell morphology, the variation in valve size detected among sites could also result from genetic differences. Allozyme analyses using five polymorphic loci identified a maximum of 13 multilocus genotypes (MLG) in MA3 at a single sampling occasion, with a total of 20 MLG identified within one inundation period (Martins et al., in preparation). On the other hand, in JAB only one MLG was identified throughout an inundation period (Adolfsson et al., submitted). No such data are available for MF4. Baltanás & Geiger (1998) associated variations in valve length and height with the presence or absence of males in populations of *E. virens*. Under the assumption that male presence guarantees enhanced levels of genetic variability, they found no effect of genetic diversity on valve heterogeneity.

Our examination of the limb chaetotaxy revealed sporadic deviations from the published progressive appearance of certain setae (Smith & Martens, 2000). However, the basic ontogenetic pattern was in agreement with that previously described. So far, no morphological differences have been found among *E. virens* females with contrasting reproductive modes, despite the application of advanced techniques of Scanning Electron Microscopy and histological preparations (R. Matzke-Karasz & R. Symonova, pers. comm.). Our microscopical investigation corroborates the absence of morphological differentiation. Such differences have been suggested for other ostracod species. Rossi et al. (2007) found that sexual *H. incongruens* females differed from asexual relatives in a morphological trait, i.e. the

presence or absence of a lamella hyalina on the posterior margin of the left valve.

Eucypris virens: population structure and dynamics

Eucypris virens was one of the first ostracod species present, and maximum densities were comparable to those in previous reports (13–200 ind. m⁻²; Baltanás, 1994). Apart from the comparable early appearance and maximum densities, populations strongly differed in many other aspects, like number of cohorts, density fluctuations and persistence. In contrast with previous designations of the species as being univoltine (Baltanás, 1994), we observed two distinct hatching peaks in the two Spanish sites. In MA3, a second hatching event may have been triggered by a temporary desiccation of the site in December. This situation exemplifies the role of diapause eggs buried in the sediment for the persistence of a population under extreme conditions. In MF4, a second hatching event occurred without desiccation after ca. 7 months of hydroperiod. This second hatching period could have resulted from subitaneous eggs produced by the first adults or from resting eggs buried in the sediment.

Otero et al. (1998) showed that in *E. virens* the production of subitaneous eggs may be induced by short photoperiod conditions, depending on clonal ecological requirements. A second generation is therefore possible from immediate hatching eggs produced by the first cohort if hydroperiods are sufficiently long. Discontinuous hatching may also reflect the variation among lineages in hatching phenologies (Rossi & Menozzi, 1990; Rossi et al., 1996; Martins et al., 2008). In the field, multiple hatching peaks may also arise from the discontinuous filling of the pond. Water level rises resulted in water surface expansions later in the season both in MA3 and MF4. Although resting egg concentrations are highest in the deepest part of the pond, eggs are also present in the sediments of more shallow areas (De Stasio, 1990). In fact, we hypothesize that temporary ponds may impose a selection pressure favouring a spatially and temporally heterogeneous deposition of resting eggs which would ensure a diversified hatching response, advantageous in highly stochastic environments (Philippi & Seger, 1989).

Differences in hatching phenologies may partly explain the observed variations in overall *E. virens* densities among and within sites. Although the physico-chemical environments displayed strong temporal variations, they are unlikely to have imposed a severe selection pressure on the *E. virens* populations. *E. virens* is a cosmopolitan species complex with broad ecological tolerance (Baltanás, 1994; Meisch, 2000). In MA3, seasonal variations in salinity followed water level fluctuations, and although high they remained within the range that can be tolerated by the species complex (De Deckker, 1981). Water temperatures varied from 3 to 20°C, whereas populations are known to persist also at higher temperatures (Gülen, 1985). Also changes in pH are unlikely to limit survival in the studied ponds (pH range: 6.5–8.6). Short desiccation periods of 1 or 2 weeks, such as those observed in MA3 may sometimes be tolerated by juvenile and adult *E. virens* buried in the wet mud. This capacity has previously been observed for other ostracod species (Delorme & Donald, 1969; Horne, 1993).

Biotic interactions may have been more important determinants of population dynamics in our study systems. Bottom-up control of population densities is unlikely, because the ephemeral nature of the environment prevents communities from reaching saturation levels. Indeed, the density of competitors increased within the season, but food items (periphyton, algae and detritus) were generally abundant also towards the end of the growing season. Predation, on the other hand, is probably more important, at least in the ponds with a relatively long hydroperiod (MA3 and MF4). Here, the richness and density of macroinvertebrate predators (e.g. Odonata, Platyhelmintha and Heteroptera) increased as the season progressed, and this coincided with a decline in the density of *E. virens*. These data are the first indications for a top-down control of ostracods in temporary ponds. Previous studies have already reported an impact of fish predation on ostracod populations and communities in permanent water bodies (Bennett et al., 1983; Gibbons, 1988).

In small water bodies, the concentration of ostracods and other biota may covary with water level fluctuations and associated changes in surface area (Williams, 2006). This is particularly relevant in MA3, whose dimensions varied within one season from few square metres to 5000 m². Still, the

strongest density fluctuations were observed in JAB, a site with relatively minor changes in water surface, and at least at the second sampling occasion predator richness and density were very low. *E. virens* densities exceeded 100 ind. s.u.⁻¹ at three occasions, and the species was not present in the hand net samples at two other occasions (visits 2 and 5). This site consists of a grassy marginal area (a stretch of a ditch) which is connected to a deeper central area mainly covered by reeds. At each sampling occasion, animals were collected at four randomly chosen locations, and this sometimes included the marginal area (visits 1, 3 and 4) and other times not (visits 2 and 5). The absence of *E. virens* in the later two samples indicates a strong microhabitat preference (during visit 2 living animals were retrieved from the ditch area). Similar microhabitat preferences have been reported for *E. virens* (Griffiths, 1995, as *E. lilljeborgi*, pers. comm.). These preferences can be associated with a heterogeneous distribution of predators as well as food resources (Benzie, 1989; Uiblein et al., 1994).

Male *E. virens* were observed only in MF4. The male:female sex ratio increased over time from 1:4.5 to 1:1.0. Assuming that this population is fully sexual, the progressive evolution of a female-biased sex ratio to an unbiased sex ratio might result from differential male and female hatching phenologies or distinct survival between sexes. This last possibility has been discussed for Ostracoda (Abe, 1990; Cohen & Morin, 1990). Differential mother investment in male or female offspring is also possible (see Fisher, 1930; Godfray & Werren, 1996), but there are no indications for this in *E. virens* based on laboratory experiments (Vandekerkhove et al., 2007; Martins et al., 2008). If we assume this population is of mixed reproductive mode, sex ratio changes can be explained by a difference in the hatching response of sexual and asexual resting eggs, or by a differential survival of sexuals and asexuals. Martins et al. (2008) observed that *E. virens* resting eggs collected in bisexual populations hatched on average later and more asynchronously than resting eggs collected in asexual populations. However, the difference was limited to a few days, and cannot explain the gradual change in sex ratio that we observed over a period of ca. 7 months. Total population density decreased as the fraction of males increased, suggesting that sex ratio changes are primarily due to unbalanced

survival rather than to biased hatching. Sexuals are, in general, regarded as better competitors, except perhaps in homogenous or biological poor environments (Glesener & Tilman, 1978; Bell, 1982), but empirical support is lacking in ostracods. Chaplin (1993) observed that females gradually gained dominance over males in an Australian lake population of *Candonocypris novaezelandiae* (Baird, 1843). The associated decrease in the MLG diversity strongly suggested a replacement of sexuals by asexuals. Such an event is likely to occur in relatively stable environments, whereas more stochastic environments, like temporary pools, provide an ecological advantage to adaptable lineages, i.e. sexuals (Fluctuating Selection: Maynard Smith, 1980; The Tangled-Bank hypothesis: Ghiselin, 1974; Bell, 1982).

Summarizing, *E. virens* population dynamics appear to be determined by a variety of factors. Population build-up is fostered by emergence from the egg bank. This process can be gradual or discontinuous depending on the bathymetry of the water body, precipitation pattern and possibly also the diversity of lineages. Total population size appears to be limited by top-down control, at least in long hydroperiod sites where macrophytes and macroinvertebrate communities have time to develop. The concentration of animals on the other hand is also associated with changes in the water level, and may strongly vary among habitat patches. The proportion of males, and presumably also that of sexuals, was found to increase within a single inundation period, but monitoring of more sites and different growing seasons is needed to assess the relevance of habitat stochasticity for the persistence of sexual *E. virens* and sexuality in general.

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