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REVIEW

Freshwater ostracods as environmental tracers

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Abstract This paper revises the response of freshwater ostracods to different environmental conditions and anthropogenic impacts, with a worldwide overview of the potential use of these microcrustaceans as bioindicators and several examples of applications in different scenarios. The development of either a single species or an ostracod assemblage is influenced by physical–chemical properties of waters (salinity, temperature, pH, dissolved oxygen), hydraulic conditions, bottom grain sizes or sedimentation rates. In addition to population and community changes, morphological and geochemical changes can also be detected in the ostracod carapace, which serves as a tracer of the water quality. All these features permit to delimit the spatial effects of urban sewages, mining effluents, agricultural wastes, watershed deforestation or road building. These data are the basis for the palaeoenvironmental reconstruction of cores, with an interesting application to archaeology. In addition, favourable results of recently developed bioassays, coupled with an important variability of local assemblages under changing conditions in both waters and sediments, suggest that these microcrustaceans may included between the most promising sentinels groups in freshwater areas. These microcrustaceans show high sensitivity to pesticides, herbicides, heavy metal pollution and oil inputs.

Keywords Anthropogenic impact · Biotechnology · Environmental parameters · Ostracods

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Introduction

Numerous papers have analyzed the importance of freshwater ecosystems as an essential part of human cultures. Although they occupy only about 1 % of the Earth's surface, both lotic and lentic environments are central to the society. Nevertheless, they are being subjected to unprecedented levels of human disturbance with variable incidence on waters, sediments and biota (Saunders et al. 2002).

Different groups of organisms have been proposed as bioindicators and/or biomonitors (see Rinderhagen et al. 2000 for distinction) to test these changes. Among the macrofaunal organisms, fishes (Lu et al. 2009), mussels

(Angelo et al. 2007), gastropods (Piyatiratitivorakul and Boonchamoi 2008) or plants (Akguc et al. 2010) are usually used for this purpose. In addition, several meiofaunal groups are also included as sentinels of human-induced changes in these freshwater environments, such as diatoms (Rimet and Bouchez 2011) or nematodes (Zhu et al. 2001).

In this last category, different studies have demonstrated the importance of some microcrustaceans (e.g. ostracods) as keystone species on production and community metabolism of micro- or mesocosm freshwater beds, playing an interesting role in determining the structure of these systems (Lawrence et al. 2002). In these microenvironments, ostracods compete with oligochaetes and amphipods for food sources (Modig et al. 2000) and all these groups are predated by fishes or gastropods.

This review attempts to analyse the potential of ostracods as environmental tracers in recent freshwater ecosystems. As ostracods occur in every aquatic environment, they can be applied as bioindicators when other groups cannot be used (e.g., groundwaters, stagnant and temporary waters). Results can be applied to both evaluations of biotic changes produced by anthropogenic activities or palaeoecological/archaeological interpretations using sediment cores.

Freshwater ostracods and environmental parameters

Correlation between different environmental variables and ostracod species has been demonstrated in numerous field investigations. A brief review is given below.

Water and ostracods

Salinity

Salinity is considered a major factor regulating aquatic community structure in freshwater environments, especially in hydrologically closed lakes and wetlands, although there are no simple relationships between ostracod faunas and this variable. Some euryhaline species are found in limnic waters but they can inhabit even hypersaline environments, whereas others (*Ilyocypris bradyii*, *Candona candida*, *Fabaeformiscandona levanderi*, *F. protzi*, *Herpetocypris reptans*) are limited to salinities down to 6 ‰ (see Fig. 1).

Temperature

Seasonal or depth-water differences of temperature may explain important changes on both ostracod density and diversity. Low temperatures seem to favour the development of some species, e.g., *Candona neglecta* or *Darwinula stevensoni* (Martens and Tudorancea 1991; Külköylüoglu and Yilmaz 2006), while other species (*Isocypris beauchampi, Cyprideis torosa*, *Cyclocypris ovum*) increase their abundances with higher temperatures (Rieradevall and Roca 1995). A third group (e.g., *Cytherissa lacustris*, *Heterocypris incongruens*) presents a relatively broad tolerance to temperature changes (Danielopol et al. 1990; Külköylüoglu 2004).

Oxygen-dissolved concentrations

Some species (e.g., *Darwinula stevensoni*) are very sensitive to oxygen depletion, whereas others that live in shallow muddy ponds may tolerate low oxygen concentrations for short time periods (e.g. *Candona candida*, *Paracyprideis fennica*, *Cypria opthalmica* or *Heterocypris incongruens*) and a third group (e.g., *Heterocypris sorbyana*, *Candona neglecta*) is tolerant to hypoxic conditions (e.g., Dole-Olivier et al. 2000; Meisch 2000; Altinsacli and Griffiths 2001).

Temporal anoxic conditions cause usually marked falls in ostracod assemblage diversity levels (Rieradevall and Roca 1995). The time delay in responding to these low oxygen levels has been estimated at less than 1 month (Martin-Rubio et al. 2005).

pH

Most freshwater ostracods prefer alkaline or slightly acidic waters, although some species can tolerate a wide range of pH from 4.6 to 13 (Fig. 1) and others were found even in highly acidic waters (e.g. Fryer 1993). In general, ostracod species are absent at a pH <5, because calcium uptake for carapace calcification is difficult in acid waters (Griffiths and Holmes 2000; Boomer et al. 2006).

Nutrient levels

Some ostracod species are very sensitive to high concentrations of several pollutants. Phosphates cause high disturbances in some species of *Herpetocypris*, whereas the amount of nitrates affect remarkably to *Candona neglecta* (Milhau et al. 1997). Some species (e.g. *Ilyocypris inermis*) are absent in disturbed sites with high nutrient levels (Pieri et al. 2012).

Depth water

Although it is very difficult to obtain a statistical correlation between depth and either ostracod diversity or the abundance of individual species, some general patterns have been established in stable freshwater environments (e.g., Fig. 2). Some species (*Limnocythere inopinata*, *Darwinula stevensoni*, *Ilyocypris echinata*) are typical of

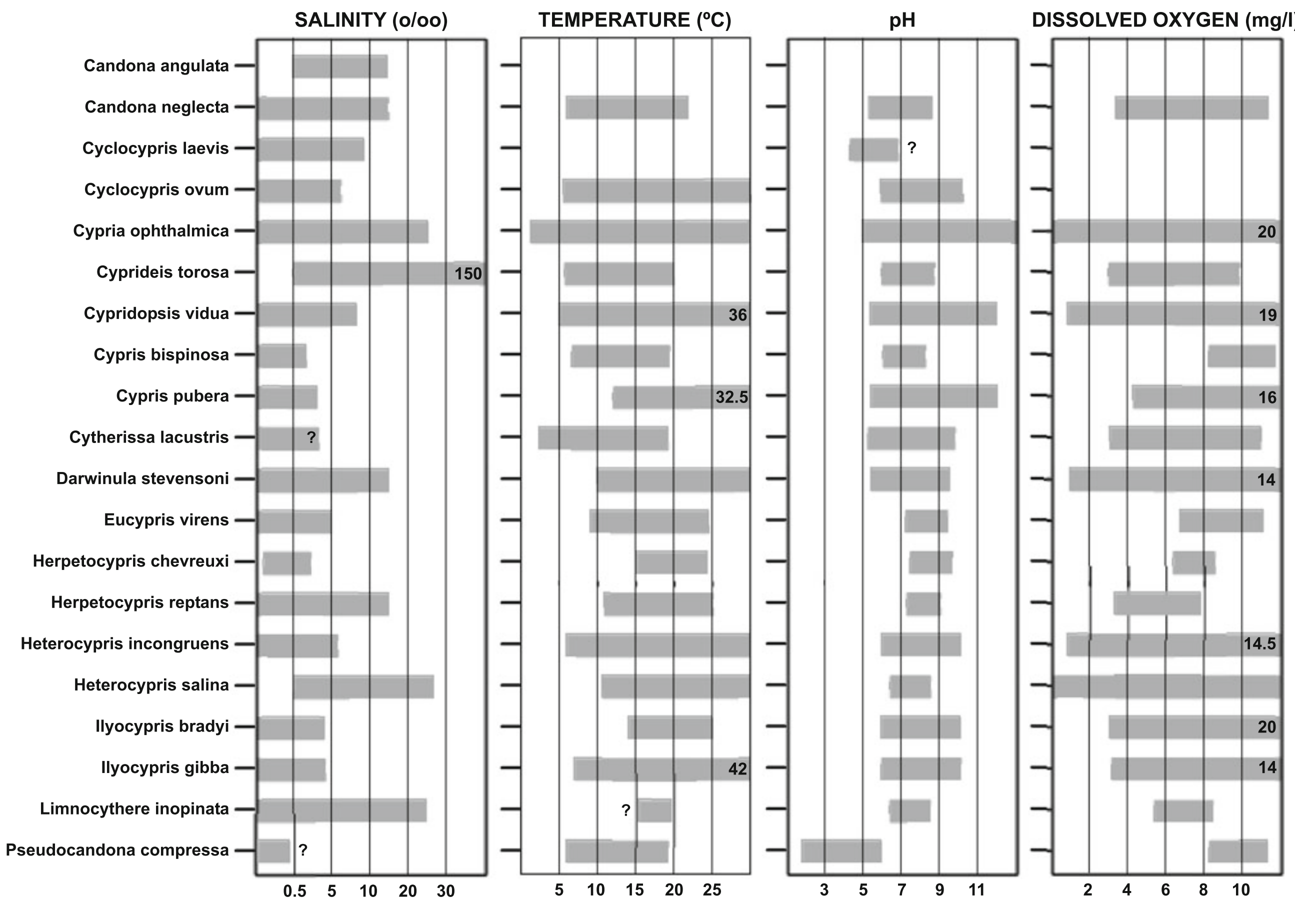

Fig. 1 Life physico-chemical conditions of twenty selected freshwater species (>100 papers revised from 1969 to 2011)

the benthos of shallow areas, whereas *Candona angulata, C. candida, Cryptocandona reducta, Cypria ophthalmica, Cyclocypris ovum*, *Cytherissa lacustris*, *Potamocypris smaragdina* or *Limnocythere sancti-patricii* can be also present in the deeper benthos (>40 m deep) (Griffiths et al. 2002; Li et al. 2010).

Depth water is clearly linked to climatic changes in some areas. In temporal lakes, swimming species are dominant during wet, deep-water periods, whereas burrowing species characterize the dry, shallow-water periods and scarce individuals are found if lakes dry out completely (Curry 2003).

Hydraulic conditions

Ostracods avoid generally high water velocities by moving inside sediments or vegetation, although they may be abundant in interstitial habitats of rapidly flowing streams (Creuzé des Chatelliers and Marmonier 1993). Moreover, some species present significant positive correlation with water turbidity (Yilmaz and Külköylüoglu 2006).

In addition, the thanatocoenosis distribution may be indicative of the seasonal hydrodynamic conditions. Isolated valves of freshwater species (e.g. *Cytherissa lacustris*, *Lineocypris* sp.) are transported even 2 km seaward during high flows, whereas only marine species were found during low flows in the same area (Ruiz et al. 1998).

Species traits and habitat utilization

Long life spans, late maturity, low fecundity and low migratory ability, medium size and geometric carapace shape are species traits of ostracods living in interstitial and hypogean habitats. In permanent flowing and standing surface waters, the abundant epigean species have long life spans, various body forms, large size and some of them give parental care. On the contrary, in temporary ponds and stagnant waters, most of the species have short life spans, high migratory ability, high tolerance desiccation and are spherical or cylindrical shape in temporary ponds and stagnant waters (Marmonier et al. 1994).

The outline of ostracods carapace can provide valuable information on the stability/instability of the environment. Species of Candoninae have triangular, trapezoidal or elongate valves with accurate posterior margin in stable

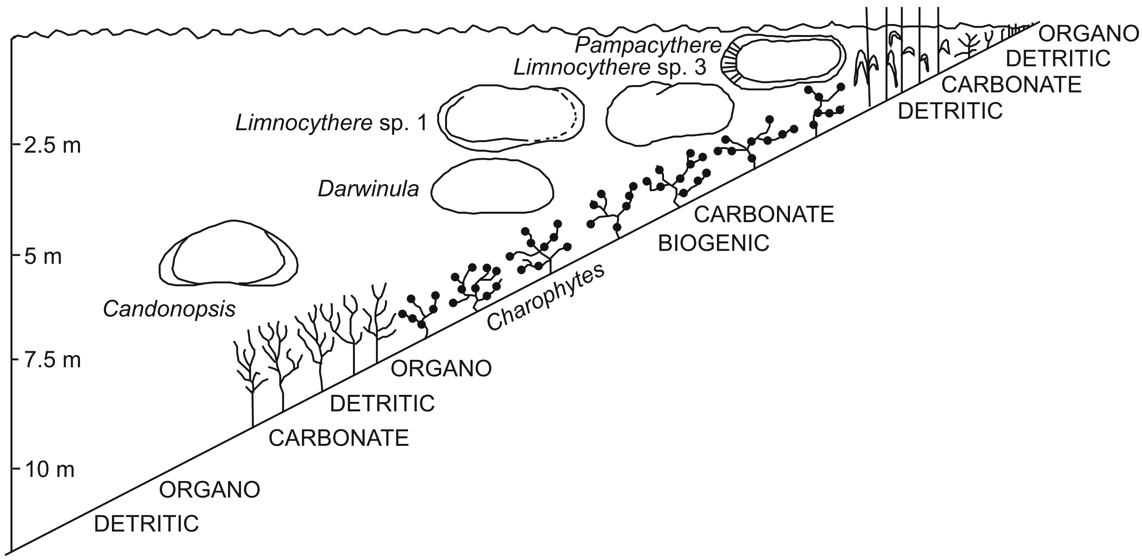

Fig. 2 Distribution of the main groups of species of ostracods related to water depth and sediment type in Lake Huinaymarca, Bolivia (modified from Mourguiart et al. 1986)

environments, whereas *Cypria* species with a sub-circular outline characterizes unstable environments (Pipik and Bodergat 2005).

Sediments and ostracods

Grain size

The influence of this factor is variable on the ostracod assemblages. In some lakes, the ostracod survival rates decreased with decreasing particle size (Donahue and Irvine 2003), whereas the effect of grain size distribution is likely to be insignificant on ostracod populations of some freshwater ponds and river biofacies (Ikeya and Hanai 1982). Some species (e.g., *Limnocythere inopinata*, *L. sanctipatricii*, *Leucocythere mirabilis*, *Ilyocypris bradyii*) usually occurs in fine-grained sediments (Lambert 1997), in contrast with the high abundances of these microcrustaceans found in coarse facies of some karstic lakes and alpine streams (e.g. Suren 1992). In addition, both this parameter and salinity changes may have a remarkable effect on the ornamentation pattern of selected species in other areas (Fig. 3: *Cyprideis torosa*).

Sedimentation rates

In some lakes subjected to a increasing deforestation around them, these crustaceans are very sensitive to high sedimentation ratios derived from the rapid erosion, with reduction in species richness up to 40 % in the shallowest areas. In these lakes, ostracods are more affected than diatoms by sedimentation (Cohen et al. 1993). This decrease has been also observed on estuarine environments, coinciding with increased sedimentation rates as a result of land modifications (Hayward et al. 2004).

The ostracod carapace as environmental tracer

Ornamentation

Surface external ornamentation has been also applied in environmental studies. *Cytherissa lacustris*, *Limnocythere inopinata* or *Cyprideis torosa* show smooth, punctuated, reticulated or noded carapaces (Fig. 4) depending on salinity range (Vesper 1975; Zhai et al. 2010) and have rounded pores in freshwater environments, whereas irregular pores are present mainly in oligohaline to hypersaline waters. Rosenfeld and Vesper (1977) proposed a graphic diagram to calculate palaeosalinities based on percentages of round, elongate and irregular sieve pores of this species, which have been used in the palaeoenvironmental reconstruction of sediment cores (Gliozzi and Mazzini 1998). Nevertheless, Keyser (2005) thinks the noding problem in *C. torosa* is mainly an osmotic control one, because the noded specimens are found in low salinity waters but also in low calcium content. According to this author, ecophenotypism in ostracod species is probably the result of a multifactorial system.

Influence of temperature cannot be omitted. The most reticulated carapaces are also the richest in Mg (Fig. 3; Carbonel and Tölderer-Farmer 1988).

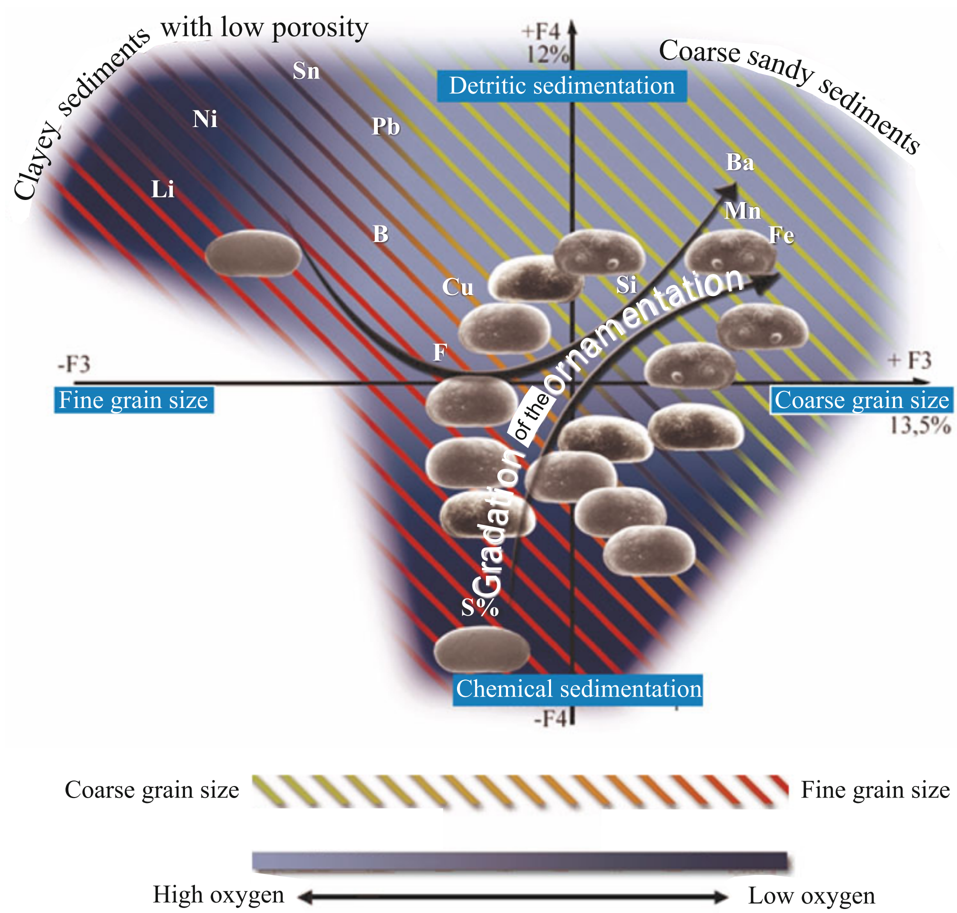

Fig. 3 Influence of the granulometry (edaphic support) on the ornamentation of *Cyprideis torosa*. Specimens have been collected from the South of Spain (Alicante province), Southwest of France (Camargue, Bouches-du Rhône) and Northwest of France (Noirmoutier Island) (modified from Bodergat 1983)

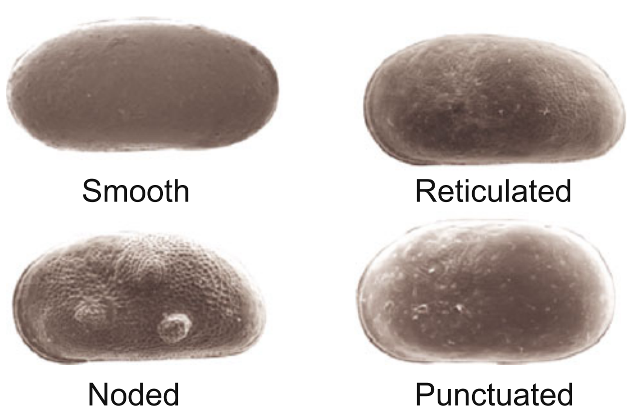

Fig. 4 Different types of ecophenotypic ornamentation on *Cyprideis torosa* (modified from Bodergat 1983)

Geochemistry

Trace elements and stable isotope geochemistry of fossil carapaces can provide very useful palaeoenvironmental informations (e.g. Jin et al. 2011). In a freshwater species, the phosphorus content (in % or ppm) of the carapace may be similar in the same environment and can change between different geographical localities, being indicative of changing geochemical conditions (e.g. Bodergat 1979).

In addition, it is important to indicate the position of the analysis in the ostracod carapace, because the percentages of an element change between internal or external zones of the same carapace (Carbonel and Tölderer-Farmer 1988). According to Rio et al. (1997), antero-posterior differences are evident and variations between inner and outer parts of the shell are less frequent. If the elements associated with mineral inclusions have a rather homogeneous distribution, those involved in biological pathway (P, Na, S, Mg, Ca) have a heterogeneous distribution.

Environmental applications

Industrial/mining wastes and urban effluents

Wastes derived from different pollution sources provoke important changes on both ostracod density and diversity. In heavily organic-polluted waters close to urban or industrial concentrations, ostracods are usually very scarce and can even disappear (Poquet et al. 2008). These effects diminish generally downstream in some rivers, with the presence of different ostracod assemblages along a gradient from high pollution towards the final "recovery" zone (Fig. 5; Mezquita et al. 1999a, b).

Mining activities produce similar effects on the ostracod assemblages, with a rapid decline of species found near the point of treated polluted underground waters (Van der Merwe 2003). Nevertheless, a part of this pollution may be eliminated by some ostracod species, such as *Herpetocypris chevreuxi* (Onderikova 1993).

Agricultural wastes

The widespread and massive use of fertilizers, pesticides and herbicides causes a decrease in ostracod richness (Rossi et al. 2003), although some species are resistant to pesticides or organic pollution (Lim and Wong 1986). Tolerant taxa are dominant in lowland springs with high nitric nitrogen contents (>800 μM) derived from a diffuse pollution of agricultural origin (Rosetti et al. 2004), whereas important increase of phosphate contents has been related to the absence of living specimens in the upper sediments of some lakes (Wünnemann et al. 2006).

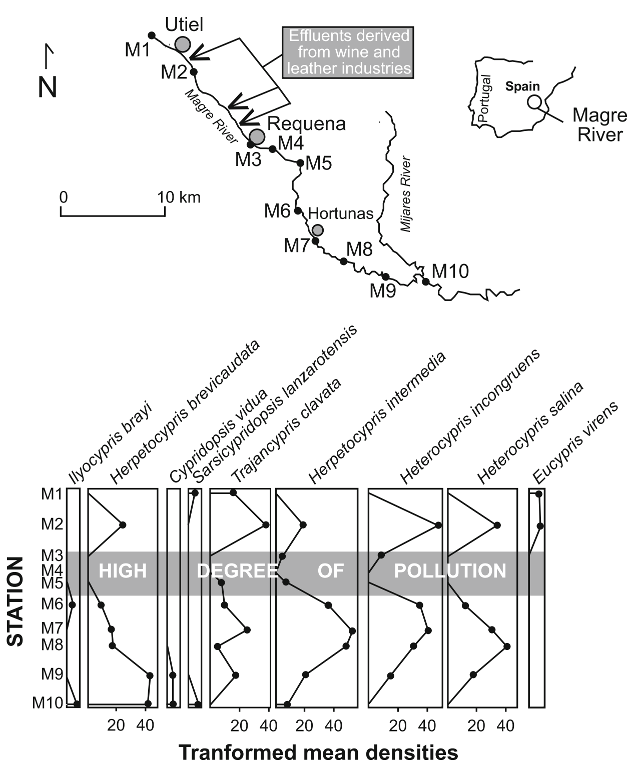

Fig. 5 Impact of industrial effluents on the ostracod populations of Magre River (Eastern Spain), with transformed average density values calculated for seven monthly field samplings (January 1995–July 1995). The scales of abundance indicate 10 ln(x + 1), where x is the mean number of individuals per m^2 (modified from Mezquita et al. Mezquita et al. 1999a, b)

Influence of other human activities

Other anthropogenic activities have also an important influence on the ostracod species richness. Watershed deforestation and road building, together with municipal and industrial discharges, result in sediment inundation of lacustrine habitats and decreasing ostracod diversities (up to 30 %) at the high-disturbance sites (Alin et al. 1999).

The impact of catchment land uses is also detected. In several small streams, few individuals and taxa occupied the hyporheic zones of streams draining hill-country catchments under pasture, which leads to hill-slope slumping, channel narrowing, an increasing erosive forces in the stream channel and reduces the habitable hyporheic zone (Boulton et al. 1997).

Palaeoenvironmental applications

Palaeoenvironmental trends

Numerous studies have been focused on palaeoenvironmental reconstructions based on a multivariate analysis (stratigraphic units, mineralogical data, macro- and microfaunal assemblages, isotopic trends) of cores collected in freshwater environments, including the ostracod analysis (De Deccker and Forester 1988; Lord et al. 2011). Autoecological stratigraphic analysis of ostracod assemblages is especially interesting in lakes where the persistence of local populations is often threatened by disturbance and changes in both water availability and quality. In sub-recent studies carried out on sediment cores (e.g. Ramdani et al. 2001), changes in the ostracod assemblages or population age structure have been related to the introduction of new predators (Fig. 6a), construction of a freshwater drainage channel (Fig. 6b), or increase of the water management for agriculture (Fig. 6c).

On the other hand, alternances of both brackish/marine or freshwater associations allowed the recognition of salinity variations that could be related to Pleistocene sea-level changes (Gliozzi and Mazzini 1998), salinity and lake-level variations (Holmes et al. 2007) or palaeogeographical reconstructions (Ruiz et al. 2004). These assemblage changes, together with isotopic studies applied to ostracod carapaces, are very useful to reconstruct climatic changes (warm/cold phases), depth-water variations or hydrological/hydrochemical conditions (e.g. Anadón and Gabàs 2009).

Some morphological features (size, shape) of ostracod species have been used to reconstruct palaeoenvironmental conditions. Occurrence of large sized and geometric ostracod carapaces indicates a stable environment and the presence of different morphologies permits to attest rift

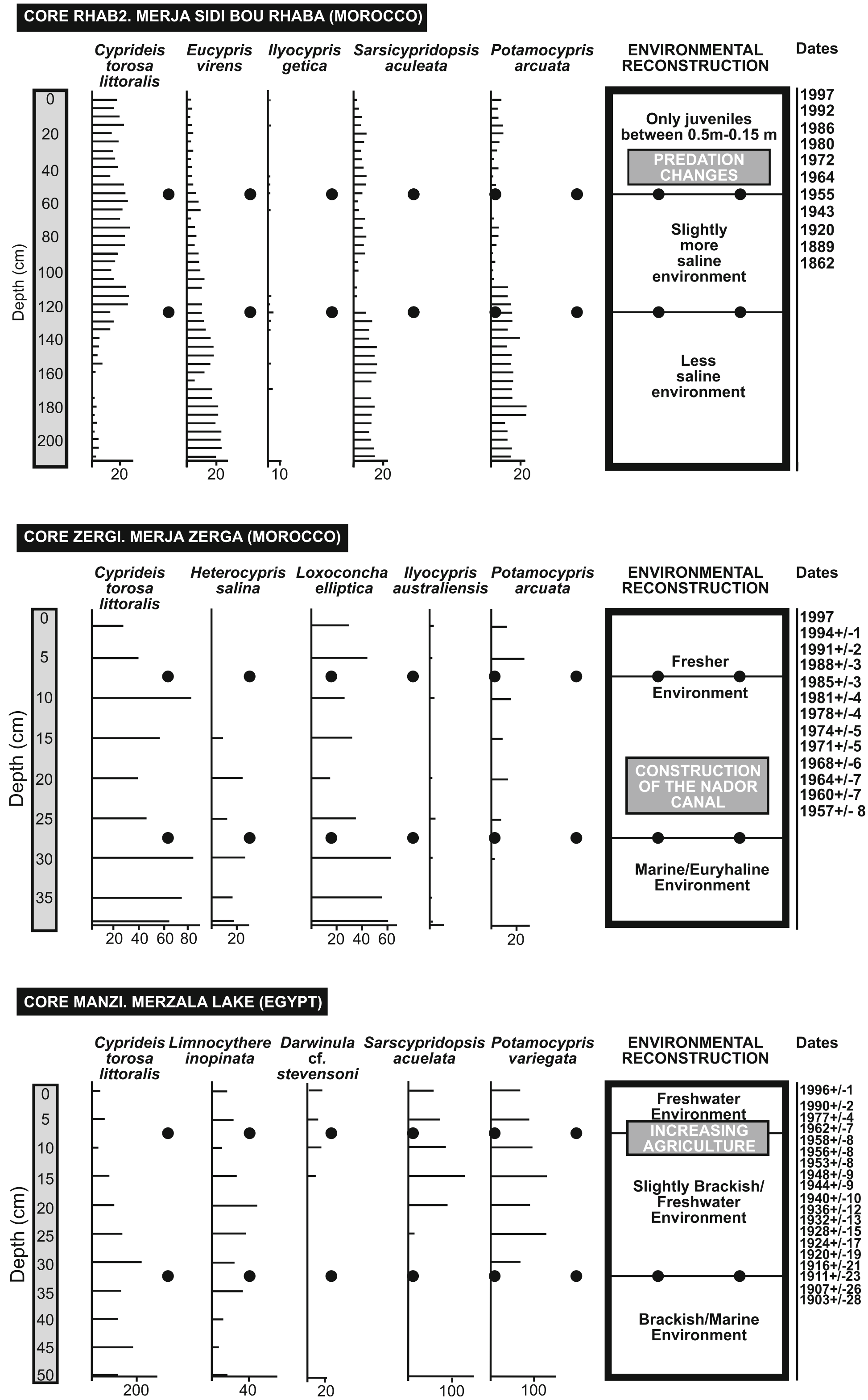

Fig. 6 CASSARINA Project. Evolution of ostracod assemblages and palaeoenvironmental reconstructions of three cores collected in three North African wetland lakes (modified from Ramdani et al. 2001). *Bars* represent the number of individuals in 10 cm^3 sediment

activities, without any variations of the sedimentology (Huguenev et al. 1999).

Nevertheless, Holmes (1996) indicated a number of problems related to: (1) methods used for extraction of ostracod shells from sediment and their subsequent cleaning; (2) *post-mortem* diagenesis and alteration of the shell; (3) complications with the calcification mechanism; (4) spatial and temporal variability in shell composition;

(5) the ecological tolerances of individual species; and (6) the relationships between shell chemistry and palaeohydrology.

Palaeoclimatic reconstructions

Lacustrine ostracods can be used as palaeoclimatic tracer (mainly for Quaternary). Isotopic analyses (^{18}O and ^{13}C) of ostracod carapaces can help to the understanding of regional events (Bahr et al. 2006; Mischke 2010) or possible exchanges between lakes and seas (Roy et al. 2011). These studies can be used in combination with protein dating by amino-acid racemization to provide valuable information on palaeotemperatures.

Environmental archaeology

These previous applications are very useful in archaeology, with a usual collection of ostracods together with plant remains, fragments of molluscs, foraminifera, bones, pollen and/or spores (Centre for Archaeology Guidelines 2002; Bates et al. 2008). Griffiths et al. (1993) provide a useful summary of sampling, preparation and identification techniques.

The shell chemistry and isotopic composition can be used even to reconstruct the climatic variations related to different periods of old cultures (e.g. Maya; Escobar et al. 2010). Changes from marine to freshwater ostracod assemblages permit to infer the end of activity in old harbour channels (Mazzini et al. 2011), whereas some ratios (Mg/Ca, Sr/Ca) have been utilised for understanding the evolution of prehistoric civilizations (Fig. 7: Hohokam culture, Arizona; Palacios Fest, 1997).

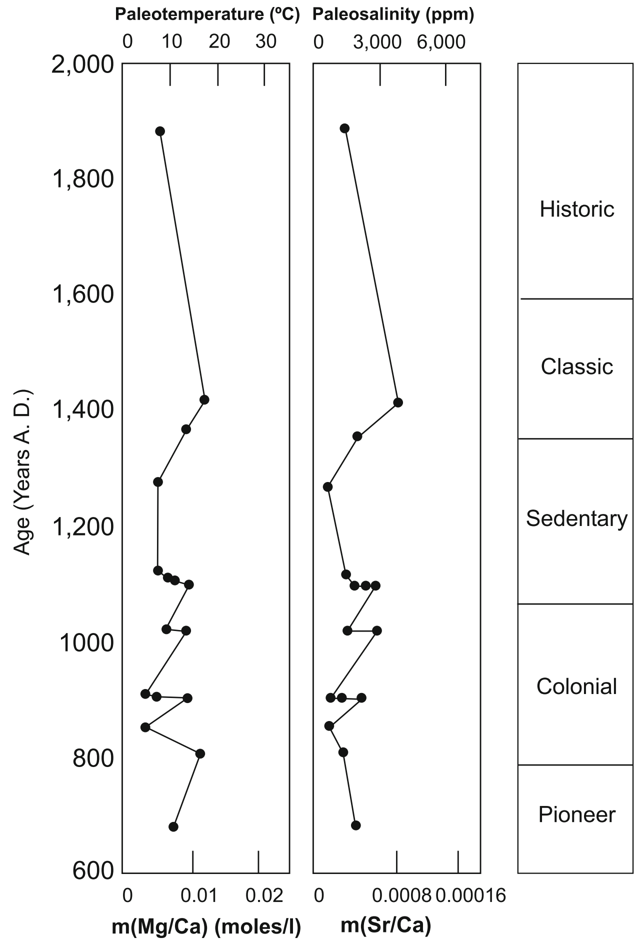

Fig. 7 Trace element palaeoenvironmental reconstruction of Hohokam canals history (Phoenix Basin Hohokam Canals, Arizona) based on ostracod shell chemistry of *Limnocythere staplini* (modified from Palacios-Fest 1997)

Laboratory studies: an additional promising approach

In numerous laboratory studies, freshwater ostracods present hopeful perspectives as biomonitors of stress conditions, giving information about the environmental state by means of the correlation between the presence/disappearance of some species, the total abundance or the population dynamics and the pollutant levels.

Herbicides and pesticides

Several experiments have analyzed the effects of variable doses of either herbicides or pesticides on different ostracod species (Fig. 8). These short studies (24–96 h of exposure time to toxins, in most cases) indicate that these microcrustaceans are excellent bioindicators, with a sensibility higher or similar to copepods, amphipods, cladocerans, crayfishes or prawns (Australian and New Zealand Environmental and Conservation Council 2000). Low doses of herbicides (e.g., dioxin) or pesticides (e.g., DDT, mexacarbate) cause an initial accumulation in the soft parts (Matsumura 1977), whereas increasing concentrations of other pesticides provoke intoxication, immobilisation or even mortality in the populations of *Heterocypris incongruens*, *Cypretta* spp., *Eucypris* sp. or *Cypridopsis* spp. (see Table 1 for a review).

Heavy metals

A "culture/maintenance-free" microbiotest (6-day Ostracodtoxkit F^{TM}; Chial and Persoone 2002a) has utilized the freshwater species *Heterocypris incongruens* (Table 2), indicating that the ostracod mortality in Zn-polluted soils was a result of the (non-soluble) toxicants bound to the solid-phase particles, rather than of those that had dissolved in the water phase (Chial and Persoone 2003). More recently, this test is used as part of a battery of bioassays to

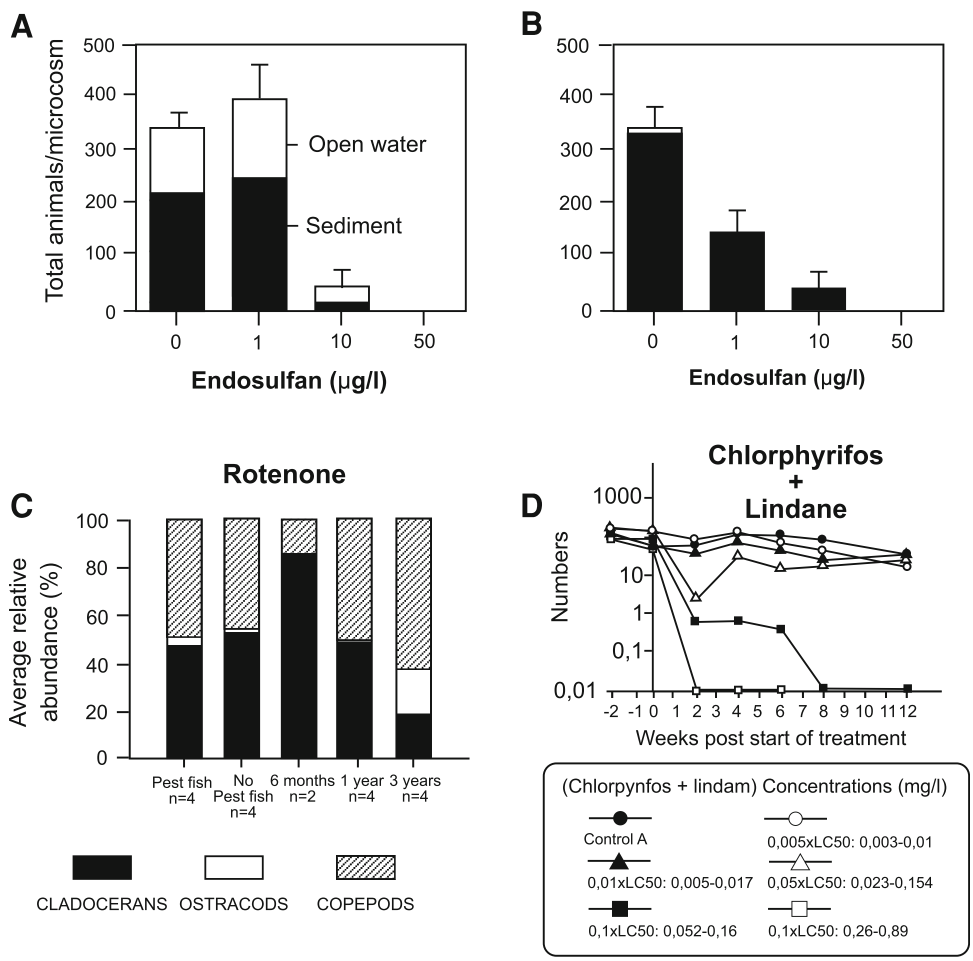

Fig. 8 Impact of the organochlorine pesticide Endosulfan (**a**, **b**), the broad-spectrum insecticide Rotenone (**c**) and two combined insecticides (**d**: Chlorphyrifos/Lindane) on the ostracod populations. **a**, **b** Effect of endosulfan on total numbers ± SE of ostracods in microcosms of *Eucypris* sp. and *Cypretta* sp. 10 weeks after initial application in southwestern Victoria, Australia (modified from Barry and Logan 1998). **c** Mean relative abundances (%) of zooplankton collected in sweep-nets at five treatment levels in 18 orchard ponds in the Motueka region, New Zealand (modified from Blakely et al. 2005). **d** Changes in number of ostracod taxa expressed as the geometric means of the numbers counted by treatment level of Ostracoda (modified from Cuppen et al. 2002)

characterize the toxicity of fluvial sediments (e.g., Wang et al. 2009).

An additional bioassay test applied to *Cypris subglobosa* to measure the toxicity of 36 metals and 12 reference toxicants reveals that osmium was found to be the most toxic in the test while boron, the least toxic (Khangarot and Das 2009). Increasing concentrations of Cu and higher acidity in waters increase the mortality on populations of this species (Khangarot and Ray 1987). This metal and Cd were included between the most toxical to *Stenocypris major* in other toxicological studies (Shuhaimi-Othman et al. 2011).

In these polluted waters, some freshwater species (i.e. *Chrissia halyi*) may survive, being the excretion a tolerance mechanisms for survival (Prasuna 1994). The efficiency of this mechanism decreases if the nominal concentration of lead in water increases (Prasuna et al. 1996). Consequently, it is necessary to analyse the relation between the metal contents of both waters and sediments and the ostracod abundance and diversity.

Oil contamination

The application of this 6-day Ostracodtoxkit™ microbiotest to oil-contaminated sediments shows that *Heterocypris incongruens* is more sensitive than the amphipod *Hyalella azteca* to contaminated sediments collected 6 and 21 weeks after oil was applied to the experimental plot. In addition, the precision of this ostracod test becomes higher or similar to that of the *Hyatella* test (Blaise et al. 2000; Chial et al. 2003a). In these time-related experiments, it is interesting to contrast the ostracod–amphipod results with other solid-phase bioassays such a Microtox® or ASPA, which use the luminescent marine bacterium *Vibrio fischeri* and the unicellular freshwater chlorophyte *Pseudokirchneriella subcapitata* as test organisms, respectively (Blaise and Ménard 1998).

More recently, other species (e.g. *Stenocypris hislopi*, *Cypretta seurati*; Tamura et al. 2011) have been used in acute lethality tests of biodegradable lubricants. The longevity of *C. seurati*, physically more active, was strongly

Table 1 Effects of pesticides (P) and herbicides (H) on the ostracod populations

Name	Formula	Type	Ostracod species	Time study (h)	Toxic dose (μg/l)	Effect	Reference
Cadmium chloride	$CdCl_2$	P	*Cypridopsis* sp.	96	190	Mortality	Fennikoh et al. (1978)
DDT	$C_{14}H_9Cl_5$	P	*Heterocypris incongruens*	24	0.04–1.74	Accumulation	Matsumura (1977)
			Heterocypris incongruens	24–48	100–5,000	Mortality	Khudairi and Ruber (1974)
Dioxin (2,3,7,8-TCDD)	$C_{12}H_4Cl_4O_2$	P	*Heterocypris incongruens*	24	0.002–0.2	Accumulation	Matsumura (1977)
Diquat	$C_{12}H_{12}N_2$	H	N.d.	48	19–46,600	Two copepods were the most sensitive and an ostracod and a cyclopoid copepod the least	Australian and New Zealand Environmental and Conservation Council (2000)
Endosulfan	$C_{12}H_4Cl_4O_2$	P	N.d.	48–96	0.9	Most sensitive were copepods and ostracods	Australian and New Zealand Environmental and Conservation Council (2000)
			Cypretta sp.; *Eucypris* sp.	1680	0–50	Elimination of the total population at high concentrations (10–50 μg/l)	Barry and Logan (1998)
Endrin	$C_{12}H_8Cl_6O$	P	N.d.	48–96	0.5–74	Ostracods and prawns as the most sensitive groups	Australian and New Zealand Environmental and Conservation Council (2000)
Formaldehyde	CH_2O	P	*Cypridopsis* sp.	1–96	236–4,760	Intoxication	Bills et al. (1977a)
Lindane	$C_6H_6Cl_6$	P	*Heterocypris incongruens*	24	2.07–6.9	Accumulation	Matsumura (1977)
			N.d.	48–96	3.2–1,100	High to moderate toxicity. Ostracods were the most sensitive group.	Australian and New Zealand Environmental and Conservation Council (2000)
Malachite green	$C_{23}H_5N_2$	P	*Cypridopsis* sp.	6–96	2,490–8,570	Mortality	Bills et al. (1977b)
Malathion	$C_{10}H_{19}O_6PS_2$	P	N.d.	48–96	1.4–6.2	Inmobilisation. The most sensitive groups were cladocerans, ostracods and copepods	Mayer and Ellersieck (1986)
Mexacarbate	$C_{12}H_{18}N_2O_2$	P	*Heterocypris incongruens*	24	3.71–5.4	Accumulation	Matsumura (1977)
Molinate	$C_9H_{17}NOS$	H	N.d.	48–96	180–33,200	Ostracods and cladocerans were more sensitive than crayfishes and praws	Australian and New Zealand Environmental and Conservation Council (2000)
Trifluralin	$C_{13}H_{16}F_3N_3O_4$	H	N.d.	48–96	37–2,200	Inmobilisation	Australian and New Zealand Environmental and Conservation Council (2000)

Table 1 continued

Name	Formula	Type	Ostracod species	Time study (h)	Toxic dose (µg/l)	Effect	Reference
3-Trifluoromethyl-4-nitrophenol	$C_7H_4F_3NO_3$	P	*Cypridopsis* sp.	1–96	19,000–117,000	Inmobilisation	Hansen and Kawatski (1976)
Triflumuron	$C_{15}H_{10}ClF_3N_2O_3$	P	*Cypridopsis* sp.	336–504	6	Lower abundance	Ali and Lord (1980)

Table 2 Contrasts of the Ostracodtox kit™ miocrobiotest with other well-known microbiotests under different experimentally induced pollution

Test	Contrast	Effects	Reference
Hatching time, size of the cups of the multiwell test plates, feeding of the rest organisms prior to the test, amount of supplemental algal food, volume of sediment and duration of the test	*Hyalella azteca* (amphipod) solid-phase test	Test protocol for a 6-day assay in 12-cup multiwell plates with ten organisms per cup and three replicates. Calibrated sand as reference sediment. Mortality and growth of the ostracods determined after 6 days incubation at 25 °C in darkness	Chial and Persoone (2002a)
Oil pollution	Statistical confidence intervals (95 %)	Development of new procedures: selection of a validity threshold for amount of substrate (300 µL), number of replicates (6), mortality (20 %) and good health of the test organisms (600 µm)	Chial and Persoone (2002b)
"Culture/maintenance-free" direct contact	*Hyalella azteca* (amphipod)-*Thamnocephalus platyurus* (crustacean)-*Raphidocelis subcapitata* (microalgae)	Complementary (nonredundant) information provided by the four tests	Chial and Persoone (2002c)
"Direct contact" toxicity determination	Springtail *Folsomia candida*	Ostracod test species sensitive as or, in several samples, even more sensitive than the springtails. Ostracod mortality as result of the (non-soluble) toxicants bound to the solid-phase particles	Chial and Persoone (2003)
"Culture/maintenance-free" direct contact	*Hyalella azteca* (amphipod)-Midge larva *Chrironomus riparius*	Sensitivity quite similar between the three organisms	Chial et al. (2003a)
Oil pollution	*Hyalella azteca* (amphipod) solid-phase test	Six weeks: Higher mortality of Ostracods. Fifteen weeks: sediments still toxic to ostracods but not to *Hyalella*. Lower variation coefficients between replicas of the ostracod results	Chial et al. (2003b)

affected by water pollution, with increasing adverse effects if the oleic acid used contains Cu.

Conclusion

Freshwater ostracods are excellent bioindicators of the surrounding physical–chemical conditions, with a remarkable response to variable salinities, water depth, temperature ranges, or pH. The ostracod carapace reflects faithfully these variations, with an interesting correlation between the water properties and both the external ornamentation and geochemical composition. Moreover, oxygen depletion, high sedimentation ratios or vigorous bottom current velocities are unfavourable factors for the development of these microcrustaceans.

The application of this dataset is very useful in palaeoclimatic reconstructions and archaeology, where the ostracod contributions are used together with additional sedimentological, geochemical or dating analyses.

Finally, in recently developed microcosm experiments, these microcrustaceans showed similar or higher sensibility to herbicides, pesticides, oil spills or heavy metal pollution than other traditional groups (like copepods, amphipods, bacteria), which are used to test anthropogenic impacts.

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