Ostracods and stable isotopes: proxies for palaeoenvironmental reconstructions

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Abstract: Carbon and oxygen stable isotope distribution is widely used for palaeoenvironmental studies. In the last 30 years these geochemical techniques were also applied on ostracod shells in particular for reconstruction of non-marine settings. The aim of this paper is to provide a general overview on methods applied and results obtained by stable isotope studies. Major attention will be focused on problems and advantages related to stable isotope analyses on ostracod shells.

Key Words: Ostracods; Stable isotopes; Methods.

1. Introduction

For palaeoclimatic and palaeoenvironmental studies, both for marine and non-marine settings, the most common standard technique is the analysis of stable isotopes. The potential of these methods was recognized since the publication of the seminal papers of UREY (1947), McCREA (1950), and UREY et al. (1951).

Analyses of the stable oxygen isotopes offer the possibility to obtain information on water temperature, precipitation/evaporation ratio and therefore on chemical composition of the host water. Oxygen isotope values are influenced by temperature, salinity, $\delta^{18}O$ of the host water and by disequilibrium effects (the so-called vital effects; see below). The analysis of carbon stable isotopes enables to decipher environmental processes widely linked to climate change but includes a broad range of parameters. Carbon isotopes are controlled by temperature and salinity as well as seasonal productivity, upwelling, and exchange between atmosphere and water. The biogenic portion is controlled additionally by diet, growth rate of the shells and reproductive status (GEARY et al. 1992; HARZHAUSER et al. 2007; LENG & MARSHALL 2004).

Stable isotope techniques can be applied both to sediments and fossils, such as foraminifers, ostracods, molluscs, fish teeth, aquatic turtles (TÜTKEN et al. 2006), and other vertebrates (AMIOT et al. 2010).

Concerning ostracods the application of stable isotope analyses gained growing importance since the early eighties of the last century. Previously the potential of ostracods in this respect was not fully realized, as, for example, stated by DURAZZI (1977: 1168): "*No strong correlation between carbon isotope ratios and temperature or salinity were observed for ostracods*".

The use of ostracods in stable isotope studies takes the following advantages:

1. Ostracod valves are quite easy to be separated from surrounding sediments;
2. their low-Mg calcite carapaces are commonly well-preserved;
3. ostracods are quite abundant in marine and non-marine sediments, but are also present in terrestrial environments;
4. ostracod shells reflect the characteristics of the water in which they grew (calcium carbonate and some trace elements are directly taken from the ambient water; TURPEN & ANGELL 1971; GOURAMANIS et al. 2010);
5. ostracods moult several times before reaching the adult stage and each moult takes place in a very short period of time. Consequently, a valve reflects discrete palaeoenvironmental conditions (TURPEN & ANGELL 1971).

The high grade of ostracods' adaptability permits to apply ostracod studies on a wide range of environments such as oceans (CRONIN & DWYER, 2003), freshwater systems (VON GRAFENSTEIN et al. 1999; TÜTKEN et al. 2006), open and closed lake basins (LENG & MARSHALL 2004), brackish waters (FRENZEL & BOOMER 2005; JANZ & VENNEMANN, 2005), and saline lakes (KEATINGS et al., 2007).

2. Stable isotopes

The term "stable isotope" describes an atom with the same number of protons (and consequently of electrons) and a different amount of neutrons. These atoms are not involved in decay processes. Hence one element and its isotopes present the same electric charge, whereas the atomic weight and the physical characteristics are different. These differences lead to isotopic fractionation that has an important role in every process on earth (ITO et al. 2003).

Isotopic fractionation comports a change in the isotopic record as a consequence of chemical (e.g., calcite precipitation), biological (e.g., photosynthesis) and physical processes (e.g., evaporation and condensation). It can occur in equilibrium conditions or in non-equilibrium conditions (kinetic fractionation). The equilibrium isotopic fractionation entails that the isotopic proportion of the two fractions remain constant through the process, although the two fractions can have different isotopic proportions. The intensity of fractionation depends on temperature and on the relative mass difference among the isotopes involved. The kinetic fractionation depends on the different velocity

of reaction of the "light" isotopes in respect to the "heavy" one. Light isotopic species react faster during a chemical reaction than heavy isotopes (Bowen 1988); consequently light isotopes have a higher concentration in the final product.

In order to avoid possible confusion that isotopic abundances in non-corresponding scales can create, the stable isotope abundances are provided in per mil as deviation from a previously fixed standard. Abundances are expressed as follow (Ito et al. 2003):

$$\delta = \left(\frac{R_{sample}}{R_{standard}} - 1 \right) * 1000 \tag{1}$$

where R indicates the ratio between the abundance of rare isotope and the abundance of the abundant isotope.

As standard for carbon isotopes of all substances the VPDB (Vienna PeeDee Belemnite, the artificial correspondent to the exhausted PDB) is used and usually the calibration material is NBS 19. VPDB has conventionally a value of 0‰ and $\delta^{13}C$ value of NBS 19 relative to VPDB is +1.95‰. $^{18}O/^{16}O$ of all substances can be expressed relative to VPDB or VSMOW (Vienna Standard Mean Ocean Water); as calibration material the NBS 19 is generally used. The $\delta^{18}O$ value of NBS 19 relative to VPDB is -2.20‰ (Coplen 1994). Conventionally VSMOW is used for analyses of silicates and water and VPDB for carbonates (Ito et al. 2003).

The PDB represents the isotopic composition of a calcareous rostrum of a belemnite found in the PeeDee Formation. The SMOW reflects the composition of an oceanic water sample preserved in Vienna. It is also possible to operate a conversion between the two standards as follows (Keatings et al. 2002b):

$$\delta^{18}O_{SMOW} = 1.03091 \times \delta^{18}O_{PDB} + 30.91 \tag{2}$$

The analysis of the isotopic ratios is realized through mass spectrometry: this technique consists in the ionization of a molecule. The so obtained ions are differentiated by their mass/charge ratio and recognized through a detector (Rollinson 1993). The isotope abundances are given in relation to a standard (see above).

2.1. Oxygen isotopes

Oxygen has three stable isotopes: ^{16}O (99.763%), ^{17}O (<0.1%) and ^{18}O (0.1995%), however, for palaeoclimate studies only ^{16}O and ^{18}O are used.

Considering (1), oxygen isotopic composition of a sample is defined as following (Coplen 1994):

$$\delta^{18}O = \frac{\left[\left(\frac{\delta^{18}O}{\delta^{16}O} \right)_{sample} - \left(\frac{\delta^{18}O}{\delta^{16}O} \right)_{standard} \right]}{\left(\frac{\delta^{18}O}{\delta^{16}O} \right)_{standard}} * 10^3 \tag{3}$$

Consequently, a positive value of δ indicates that the sample is enriched in the "heavy" isotope in respect to the standard, a negative value of δ indicates an enrichment in "light" isotopes and a depletion in "heavy" isotopes in the sample.

In general, global climate is one of the main control factors for oxygen isotope values in open oceans, whereas for marginal or coastal areas local environmental changes have a great importance. Oxygen stable isotope composition in lacustrine systems, depends on the precipitation/evaporation ratio (P/E–ratio), hence on temperature and salinity, on the $\delta^{18}O$ content of the ambient water, on $\delta^{18}O$ composition of groundwater and river input and on the catchment area. When analysing biogenic material vital effects must be taken into account. When the isotopic fractionation of a mineral occurred in equilibrium (see above), its isotopic composition is influenced mainly by temperature and ambient water isotopic composition. In that case it is, theoretically, possible to apply the palaeotemperature equations to obtain information about the temperature range and hence on the palaeoenvironmental conditions. The original equation was developed by EPSTEIN et al. (1951, 1953) and later modified by other authors. Table 1 reports some of the temperature equations for the equilibrium precipitation of carbonates from solution.

EPSTEIN et al. (1951): $\quad T°C = 16.5 - 4.3\,(\delta^{18}O_c - \delta^{18}O_w) + 0.14\,(\delta^{18}O_c - \delta^{18}O_w)^2$
EPSTEIN et al. (1953): $\quad T°C = 16.2 - 4.2\,(\delta^{18}O_c - \delta^{18}O_w) + 0.13\,(\delta^{18}O_c - \delta^{18}O_w)^2$
CRAIG (1965): $\quad T°C = 16.9 - 4.2\,(\delta^{18}O_c - \delta^{18}O_w) + 0.13\,(\delta^{18}O_c - \delta^{18}O_w)^2$
O'NEIL et al. (1969): $\quad T°C = 16.9 - 4.38\,(\delta^{18}O_c - \delta^{18}O_w) + 0.1\,(\delta^{18}O_c - \delta^{18}O_w)^2$
SHACKLETON (1974): $\quad T°C = 16.9 - 4.38\,(\delta^{18}O_c - \delta^{18}O_w) + 0.1\,(\delta^{18}O_c - \delta^{18}O_w)^2$
EREZ & LUZ (1983): $\quad T°C = 17.0 - 4.52\,(\delta^{18}O_c - \delta^{18}O_w) + 0.03\,(\delta^{18}O_c - \delta^{18}O_w)^2$
ANDERSON & ATHUR (1983): $\quad T°C = 16.0 - 4.14\,(\delta^{18}O_c - \delta^{18}O_w) + 0.13\,(\delta^{18}O_c - \delta^{18}O_w)^2$
XIA et al. (1997a): $\quad T°C = 16.9 - 4.2\,(\delta^{18}O_c - \delta^{18}O_w) + 0.13\,(\delta^{18}O_c - \delta^{18}O_w)^2$
LENG & MARSHALL (2004): $\quad T°C = 16.0 - 4.14\,(\delta^{18}O_c - \delta^{18}O_w) + 0.13\,(\delta^{18}O_c - \delta^{18}O_w)^2$

Tab. 1: Temperature equations used in literature. $\delta^{18}O_c$: oxygen isotope composition of CO_2 derived by a reaction at $25\,°C$ with H_3PO_4 with respect to the mass spectrometer standard gas; $\delta^{18}O_w$: oxygen composition of the CO_2 in equilibrium at $25\,°C$ with the seawater.

Obviously these equations cannot be used uncritically. The matter is that the two main factors (P/E-ratio and $\delta^{18}O$ of the ambient water) can be influenced by a multitude of processes such as climate and environmental changes, the catchment of the lake, the $\delta^{18}O$ of precipitation and vital offsets.

Moreover, interpreting the influence of temperature on stable isotope values, attention must be paid on two processes with opposite effects that derive from the increase of temperature. An increase in temperature leads to an increase in evaporation ratio, that imply an enrichment in "heavy" stable isotopes in the ambient water with a gradient of approximately $+0.6\,‰/°C$ (DANSGAARD 1964); at the same time the equilibrium isotope fractionation between carbonate and ambient water has a gradient of $-0.24\,‰/°C$. In addition, a non-linear relationship can occur between these two processes and consequently the resulting gradient of $\sim 0.36\,‰/°C$ may be unreliable (LENG & MARSHALL 2004).

Isotopic composition of precipitation (both snow and rainfall) and the P/E-ratio have a great importance for the definition of the stable isotope composition of water in lacustrine systems. The rule of evaporation, clearly, grows in importance if the climate is arid and the lake system is closed. In general, a strong evaporation leads to enrichment in "heavy" isotopes that, because of their weight, enter in the evaporation process with more difficulties in respect to the "light" isotopes. Hence, an increase in the P/E-ratio usually leads to a depletion of $\delta^{18}O$ values (Bowen 1988). This decrement, however, can be the expression of several processes: growth of P-value or a change in the P-composition under constant temperature; decrease in temperature (and so depletion in E); increase in melt water flux caused by an increase in temperature (Jin et al. 2009). Therefore it is important to reach at least a basic level of knowledge of a lake's hydrology (as regarding, e.g., salinity, residence time, lake size), because the causes that lead to a change in $\delta^{18}O$ values can be manifold.

For the analysis of biogenic material, one must consider the influence that vital effects have on stable isotopes values. They imply disequilibriums due to pH (Keatings et al. 2002a), different precipitation rates of carbonate, metabolic fluids incorporation, growth in microenvironment with peculiar characteristic (in particular the last one can lead to significant variation within the same taxa).

2.2. Carbon isotopes

Carbon has two stable isotopes in nature and one radioactive isotope, respectively: ^{12}C (98.89 %), ^{13}C (1.11 %) and ^{14}C. The carbon isotopic composition is expressed as $\delta^{13}C$ and it is represented by the following equation:

$$\delta^{13}C = \frac{\left[\left(\frac{\delta^{13}C}{\delta^{12}C}\right)_{sample} - \left(\frac{\delta^{13}C}{\delta^{12}C}\right)_{standard}\right]}{\left(\frac{\delta^{13}C}{\delta^{12}C}\right)_{standard}} * 10^3 \qquad (4)$$

Carbon isotope values can be influenced by a multitude of factors, such as temperature, salinity, composition of inflowing waters, Photosynthesis of aquatic organisms (and hence seasonal productivity), upwelling, exchange between atmosphere and water. Additionally, when analyzing the biogenic portion diet, growth rate and reproductive status must be taken into account. The factors that may influence carbon stable isotope ratios are more diverse than that of oxygen stable isotopes. Therefore these data can be more difficult to interpret (Geary et al. 1992 and references herein).

However, $\delta^{13}C$ were successfully applied for studies regarding nutrient concentration in shallow waters and consequently about palaeoproductivity (Meyer et al. 2011). During photosynthesis, autotrophic organisms use mainly ^{12}C to build organic matter. This leads to a natural enrichment in ^{13}C of superficial waters (Bowen 1988): high values of ^{13}C indicate a tendency to eutrophism and high water turbidity, whereas low

values of ^{13}C indicate oligotrophic conditions and low water turbidity. Respiration of plants and organic production can be affected by seasonality (COLLETA et al. 2001), which can cause water stratification, especially during summer (the period that represents the highest shallow water productivity throughout the year). The stratification can result in large differences in $\delta^{13}C_{TDIC}$ (Total Dissolved Inorganic Carbon) values, reflected in the composition of carbonates precipitated at different depths in the water column. In fact, stratification leads to depletion in organic material through oxidation, which is followed by an increase of $\delta^{13}C_{TDIC}$ (LENG & MARSHALL 2004).

Upwelling effects are directly controlled by the photosynthetic activity in surface waters: phytoplankton removes ^{12}C from the ambient dissolved inorganic carbon and produce organic matter. That organic matter releases "light" carbon isotopes during its decomposition in depth, causing a depletion of ^{13}C in bottom waters. Consequently, deep waters are enriched in "light" isotopes which tend to be brought towards the surface (KRANTZ et al. 1988; GEARY et al. 1992).

The runoff of groundwater and river waters usually provide isotopically "light" carbon, however, there are several exceptions. This makes it difficult to evaluate the influence of freshwater input (in particular for palaeoenvironmental reconstructions). Obviously the water composition of these freshwaters depends on the surrounding soils, bedrock and vegetation. In absence of specific data about the catchment area $\delta^{13}C$, it is impossible to quantify the runoff effects (further information: http://www.hydrology.nl/ihppublications/149-environmental-isotopes-in-the-hydrological-cycle-principles-and-applications.html).

The effects of temperature on $\delta^{13}C$ are still not completely understood. GEARY et al. (1992) reported several studies with contrasting conclusions (direct or indirect correlation between increase/decrease of temperature and increase/decrease of $\delta^{13}C$ values). Other authors (ROMANEK et al. 1992) tried to determine carbon isotopic fractionation as a function of mineralogy, temperature, and precipitation rate and they stated that the effects of temperature are not significant for calcite fractionation.

3. Ostracods

Ostracods are small-sized crustaceans with a range in size around $200\mu m$–$32\,mm$ (PARK & DOUGLAS RICKETTS 2003). Adult specimens usually are in the size of circa 0.7–1 mm. Ostracods are adapted to a wide range of salinity (from freshwaters to marine waters), so their natural habitat spans from lakes to open oceans. There are also some rare terrestrial species. Their mode of life is mainly benthic and subordinately planctonic, they generally are vegetal and animal detritivore. Additionally, their fossil record is wide: ostracods specimens are known at least from the basal Ordovician (SIVETER et al. 2010). For these reasons ostracods are a very useful proxy for both biostratigraphic and palaeoenvironmental studies. The importance of ostracods grows

exponentially for those environments in which foraminifers are rare or lacking (e.g., in non-marine environments).

The ostracods' mineralized portion is carbonatic and mainly composed of Low-Mg calcite. In some cases the presence of amorphous calcium carbonate is reported (XIA et al. 1997a). The remainder of the carapaces is represented by organic material; an estimation of 2–15 % content in chitin and proteins for living specimens was made by Sohn (1958) and reported in XIA et al. (1997a) and BOOMER et al. (2003).

It is widely accepted that the carapace mineral composition reflects the host water chemistry, because ions that form the shells (Ca^{2+} and HCO_3^-) are directly taken from the host water. The ostracods vital cycle requires 8 to 9 moults before reaching the adult stage with a very short duration for calcification after each moult (HOLMES 1996). TURPEN & ANGELL (1971) reported that in *Heterocypris* calcification occurs within 7 hours after moulting. Nevertheless, it is necessary to take into account that the calcification moment is depending on species-specific characteristics such us the number of generations per year, the season in which a generation originates, the time past between the first instar and the adult specimen. In order to improve the stable isotope analysis interpretation, all these variables have to be considered. Hence, it is essential to gain information about ostracods' ecology. Unfortunately, ecologic data (also on living species) are far to be exhaustive, and, as reported in COHEN & MORIN (2003), the large part of ostracods' life cycles is still unknown.

The vital offset influence on $\delta^{13}C$ of ostracod valves is still not completely understood however, it seems to create systematic variations (XIA et al. 1997b). According to VON GRAFENSTEIN et al. (1999), none of the taxa they studied formed their shells in isotopic equilibrium. These disequilibriums imply a slightly positive effect on stable isotope values for almost all freshwater ostracods. In KEATINGS et al. (2002a) shifts in oxygen isotope composition are reported ranging from +2.5 to +3.0 ‰. Moreover, it is hard to define the variations caused by the vital effects on $\delta^{13}C$ with the same resolution obtainable for the $\delta^{18}O$ values, because it is difficult to separate vital effect influence from variations due to seasonality.

Consequently, it is clear that the use of ostracods entails several advantages as well as some disadvantages. However, they are considered as one of the most reliable indicators of palaeoenvironmental parameters for both marine and non-marine settings (BOOMER et al. 2003; FRENZEL & BOOMER 2005).

3.1. Methods

Valves and carapaces can contain adhering sediments, soft parts and crystals that can contaminate the isotope measurements; consequently, prior to geochemical analyses all samples must be cleaned. Although some authors have tried to set a standardized procedure (see the text below), there is not a common cleaning method. Consequently, various procedures have been applied through the years. Moreover, the biases of iso-

tope data that can be caused by chemical pre-treatment techniques are not completely understood, so it is highly recommended not to use them.

In Lɪ et al. (2007) the effects of methanol, deionised water and methanol, and hydrogen peroxide pre-treatments on ostracod shell oxygen and carbon isotope compositions are tested in comparison with untreated shells on well-preserved ostracod shells and through deionised water. Their results show that chemical pre-treatments have no observable effects on the isotopic composition of ostracod valves. They also tested the effects of valve length: according to their results, there are no variations in oxygen isotope composition, whereas carbon isotope ratio tends to be higher in shorter shells; thus a selection of valves for carbon isotope determinations has to be made with particular care.

Mɪsᴄʜᴋᴇ et al. (2008) studied the differences in the stable isotope ratio of ostracod valves that were oven-dried from tap water or from ethanol (after the sieving procedure). According to their results there are large differences in ratios among the samples washed onto a petri dish by ethanol or by tap water: for $\delta^{18}O$ values the differences are up to 3‰ (average 1‰), and for $\delta^{13}C$ values up to 1.6‰ (average 0.5‰). These authors suggest avoiding the use of tap water because it can probably entail calcite crystal precipitation on ostracod shells which may significantly alter the isotopic signature.

Following cleaning methods for both fossil and living specimens have been reported (in chronological order):

Xɪᴀ et al. (1997a): Ostracods were cultured to maturity. Individual adults were separated from soft tissues with a dissecting needle, cleaned for 15 min in 5% deionised water by 80°C, rinsed with triply distilled water on polycarbonate membrane filter and air dried in a laminar flow-hood. Small (0.1–0.3‰) and non-systematic shifts were reported.

Xɪᴀ et al. (1997b): Specimens were stored in 100% ethanol. The shells were separated from soft tissues with a dissecting needle, cleaned in 5% H_2O_2 at 80°C for 10 min, rinsed with triply distilled water and air dried on polycarbonate membrane filter in a laminar flow-hood.

ᴠᴏɴ Gʀᴀꜰᴇɴsᴛᴇɪɴ et al. (1999): Separated specimens were boiled in deionised water for one hour and stored in ethanol.

Kᴇᴀᴛɪɴɢs et al. (2002a): Deionised water and a fine brush were used for the physical removal of adhering material.

Kᴇᴀᴛɪɴɢs et al. (2002b): Analysis of a "natural culture" (living ostracods). Soft parts were cleaned using deionised water, fine brushes and entomological needles, then the valves were soaked in 5% sodium hypochlorite solution for 4 hours at room temperature and visually inspected.

Jᴀɴᴢ & Vᴇɴɴᴇᴍᴀɴɴ (2005): 1 kg of air-dried sediment was soaked in a dilute solution of hydrogen peroxide (about 3.5%). Successively the clean and well preserved valves were picked, soaked again in H_2O_2 and cleaned ultrasonically by inserting the

sample vials 3 to 5 times in the ultrasound bath for about 1 second each. Finally the samples were rinsed with distilled water for several times.

ANADON et al. (2006); ANADON et al. (2008); ANADON & GABAS (2009): The valves were separated and carefully cleaned with ethanol and distilled water, using a fine brush.

JIN et al. (2006): They tried to set a standard cleaning method for ostracod shells testing it through trace element determination. They propose as the most effective method for the removal of adhering sediments, the use of ultrasonic methanol cleaning followed by a deionised water treatment (in order to remove any remaining methanol) followed by manual fine brush cleaning. To reduce the risk of contamination from non-carbonate particles they also suggest operating a centrifuging process (500 rpm for 5 min).

TÜTKEN et al. (2006): Only clean and transparent valves were chosen, adhering sediment was removed in distilled water through ultrasonification.

DOMINGO et al. (2007): Ostracods were washed with distilled water in an ultrasonic bath and then checked in order to avoid the presence of any infilling or adhering sediment. The specimens were successively crushed in an agate mortar.

KEATINGS et al. (2007): Ostracods and other microfossils were picked with a fine brush moistened with deionised water, and then adhering sediments were removed using deionised water, fine needles and brushes.

ARP & MENNERICH (2008): The valves were mechanically cleaned, fractured, and checked with respect to overgrowth and adherent sediment by light microscopy. Only fragments free of sediment and cements were used.

LAWRENCE et al. (2008): The carapaces were rinsed with ethanol in an ultrasonic bath and dried.

JIN et al. (2009): Ostracods were initially cleaned using a brush moistened with deionised water; then the authors followed the procedure of JIN et al. 2006 (see above).

PROKOPENKO & BONVENTO (2009): The largest, cleanest and most complete shells were picked.

LAUTERBACH et al. (2011): Sediments were disaggregated in a 10% H_2O_2 solution, wet sieved through a 125 μm mesh and rinsed in ethanol before drying. Subsequently, valves have been separated and mechanically cleaned.

In summary, the selected valves or carapaces for the stable isotope analysis are as clean and transparent as possible and are often stored in ethanol. The majority of the authors use a deionised water moistened fine brush to pick up the valves and in some cases also for the removal of adhering material. An ultrasonic bath and the soak of valves in H_2O_2 or in a sodium hypochlorite solution for variable periods of time are also quite common.

A critical point for stable isotope analyses is also the quantity of analyzed material. Studies on this subject should report on the weight of the analysed material per

sample and the number of valves or carapaces. In many papers this information, however, is missing. In Table 2 the amount and weight of ostracods used is given for a selection of more recent papers.

Reference	Species	Number of valves	Total weight
Xia et al. (1997a); Xia et al. (1997b)	*Candona rawsoni*	1 carapace	not expressed
von Grafenstein et al. (1999)	*Candona* sp., *C. candida, C. lacustris, Cytherissa lacustris, Limnocythere inopinata, Darwinula stevensoni, Faebiformiscandona caudata, F. tricicatrosa, F. levanderi, F. protzi, Pisidium spp.*	1 to 12 valves	not expressed
Keatings et al. (2002a)	*Cypretta brevisaepta*	1 valve	27.8 +/- 4.2 µg
Keatings et al. (2002b)	*Candona candida, Pseudocandona rostrata, Herpetocypris reptans*	1 to 4 valves	~50 µg
Janz & Vennemann (2005)	*Aurila* spp.*, Bosquetina* sp., *Carinovalva* sp., *Cytherella* sp., *Cytheridea* spp., *Cyprideis* spp., *Hemicytheria* sp., *Henryhowella* sp., *Loxoconcha* sp., *Loxocorniculum* sp.	1 or more valves	~15 µg
Anadon et al. (2006)	*Candona neglecta*	4-14 valves	not expressed
Anadon et al. (2008)	*Fabaeformiscandona* sp., *Pseudocandona compressa, Fabaeformiscandona* aff. *F. spelaea, Fabaeformiscandona* cf. *F. breuili*	4-14 valves	not expressed
Anadon & Gabas (2009)	*Cyprideis torosa*	4-14 valves	not expressed
Jin et al. (2006)	*Psychrodromus, Qinghaicypris*	2 valves	*Psychrodromus* 210-230 µg *Qinghaicypris* 310-322 µg
Tütken et al. (2006)	*Ilyocypris binocularis*	2-4 valves	30-80 µg
Li et al. (2007)	*Eucypris inflata, Limnocythere inopinata*	3-9 valves 10 to 16 valves	not expressed
Domingo et al. (2007)	not expressed	1 carapace	not expressed
Keatings et al. (2007)	*Cyprideis torosa*	1 carapace	~70 µg
Arp & Mennerich (2008)	*Cypridea dunkeri inversa, Cypridea tumescens praecursor, Mantelliana purbeckensis, Theriosynoecum forbesi*	2-18 valves	not expressed
Lawrence et al. (2008)	*Cypridopsis vidua, Potamocypris islagrandensis, Ilyocypris biplicata*	0,5-6 valves	not expressed
Mishke et al. (2008)	*Eucypris mareiotica, Fabaeformiscandona danielopoli, Ilyocypris sebeiensis*	not expressed	not expressed
Jin et al. (2009)	*Qinghaicypris crassa*	not expressed	>100 µg
Prokopenko & Bonvento (2009)	*Candona lepnevae, Cytherissa lacustris*	2-4 valves	not expressed
Lauterbach et al. (2011)	*Candona neglecta*	up to 20 valves	15-110 µg

Tab. 2: Amount of materials.

From data analysis of Table 2, it is possible to assume that at least $30\,\mu g$ of ostracods are required in order to reach the necessary quantity of material for stable isotope analysis. Considering an average size of $1\,mm$ for adult individuals, such a quantity is represented by 2–4 valves; the amount of juvenile valves is obviously larger and in order to reach the minimum weight 4–6 valves are required. Clearly the number of valves is strictly depending on their size.

4. Conclusions

Ostracods are small crustaceans that belong to the phylum Arthropoda. They own some particular characteristics that make them suitable for palaeoclimatic and palaeoenvironmental studies. In particular it is remarkable that their moults take place in a discrete period of time and the ions that build the shells are directly taken from the host water. Consequently trough a geochemical approach it is possible to obtain reliable information about the environment in which they grew. Ostracods' importance is more evident in those settings in which there is a lack of other organisms such as foraminifers, e.g., lake systems. The most common technique in this context is the analysis of oxygen and carbon stable isotope ratio. $\delta^{18}O$ values are mainly depending on the temperature at the moment of valve calcification, on the $\delta^{18}O$ values of the host water and on salinity, whereas the $\delta^{13}C$ values are controlled by several factors such as runoff, upwelling, photosynthetic activity, diet, growth rate and reproductive status.

The importance of ostracods for these analyses is widely recognized, however, a large number of aspects are still unclear, in particular as regarding ecology and palaeoecology. In fact, diet, growth rate, vital cycles and other parameters that can bias in particular the $\delta^{13}C$ values are often very poorly known, even for living species. For example, the period of the year in which calcification occurs (and its relation to water temperature) is species specific as well as the number of moults/year, consequently it is nearly impossible to determine the vital cycle of extinguished taxa. The influence of vital effects is also a topic still widely unknown and only a few studies were made on this argument (Xia et al. 1997b; von Grafenstein et al. 1999; Keatings et al. 2002a).

It is remarkable that, although ostracods are well-used in geochemical analyses since at least 30 years, there is no commonly accepted cleaning standard for valves and carapaces and the effects of chemical pre-treatment are not completely understood. The suggestion consequently is to avoid treatments that can contaminate the measurements by using deionised water and storing samples in ethanol.

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