

Monthly variations in the shell structure of two freshwater ostracod (Crustacea) species in Karapınar Spring (Bolu, Turkey)

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Abstract: The variations of environmental conditions (T °C, pH, $\delta^{13}\text{C}$, $\delta^{18}\text{O}$, Mg/Ca, and Sr/Ca) for two ostracod species (*Psychrodromus olivaceus*, *Potamocypis similis*) were analyzed and compared to identify the controls of environmental parameters on the chemical and isotopic composition of their valves. The specimens were collected from Karapınar Spring (Bolu, Turkey) between October 2009 and November 2010. The species were observed throughout the year. *Psychrodromus olivaceus* showed relatively constant Mg/Ca ratios during the study period, whereas those of *P. similis* showed more variability. The ratio was also significantly different ($P < 0.05$) between the two species. We did not find significant correlation between water temperature and either of the species' Mg/Ca ratios. The stable isotope record for *P. similis* was very patchy, showing a small range of values consistent with the groundwater source, similar to the behavior of Sr/Ca. The Sr/Ca ratio displayed little variation throughout the year, suggesting hydrochemical stability over time, most likely in response to the groundwater source. The stable isotope data displayed pronounced variability for *P. similis* but little for *P. olivaceus*, suggesting that the former may be responding to seasonality, whereas the latter responds to groundwater.

Key words: Ostracoda, rheocene spring, isotope analyses, Mg/Ca ratio, Sr/Ca ratio

1. Introduction

Ostracods are microscopic crustaceans characterized by a hinged bivalve carapace made of low-Mg calcite and ranging in size mainly between 0.5 and 2.0 mm. Like other crustaceans, ostracods grow by successive molting (ecdysis). A new carapace may be cast and calcified within hours (Turpen and Angell, 1971), or the process may take up to a few days (Rocca and Wansard, 1997). In continental waters, they are mostly benthic, although some species are nektic and may swim around vegetation. Ostracods occur from the littoral zone to great depths, in or around springs, streams, and lakes. The carapace is the only body part that is preserved in the geologic record, where they may be abundant and well-preserved in the sediments. Today ostracods are diverse and abundant in marine and nonmarine environments. Ostracods are used for many reasons in biological, ecological, evolutionary, and paleontological studies. Therefore, their occurrence or their relative abundance may represent a valuable tool for reconstructing past environments.

Recently, many attempts have been made to understand the relationship between ostracod valve minor-element

chemistry and environmental factors (see the summary by Holmes and Chivas, 2002). The study of shell chemistry has proven useful for paleohydroclimate investigations in marine, brackish, and freshwater environments. For example, Chivas et al. (1983), De Deckker et al. (1988), Xia et al. (1997), Palacios-Fest et al. (2002), Janz and Vennemann (2005), and Tütken et al. (2006) have used trace elements for reconstructing the history of paleolakes.

The main source of Ca in the valves is that of the host water (Turpen and Angell, 1971; Rosenfeld, 1982; Majoran et al., 1999). Previous studies (see, e.g., Chivas et al., 1983, 1986; Wansard et al., 1999; Palacios-Fest and Dettman, 2001) focused on factors (e.g., temperature, salinity) affecting Mg/Ca levels in the ostracod valves. Although such studies are common for marine and brackish water ostracods (De Deckker et al., 1988; Dwyer et al., 1995; Cronin et al., 1996), studies are relatively limited for freshwater species. Among them, *Candona rawsoni* Tressler, 1957; *Herpetocypis brevicaudata* Kaufmann, 1900; *H. intermedia* Kaufmann, 1900; and *Cypridopsis vidua* (Brady & Norman, 1889) were the subjects of studies to determine the Sr and Mg content and temperature

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association (Engstrom and Nelson, 1991; Wansard and Roca, 1997; Wansard et al., 1999; Palacios-Fest and Dettman, 2001, respectively).

Stable isotopes, for example, have been successfully analyzed by Lister (1988), Schwalb et al. (1994), and von Grafenstein et al. (1999) for reconstructing lake history. The stable isotope records may show close correlations between the evaporation/precipitation pattern, temperature, water source, and productivity (Cohen et al., 2000). None of these studies come from a natural spring habitat, despite such a habitat's intrinsic value as a stable environment serving as a natural laboratory (Hynes, 1970; Glazier, 1991; Särkkä et al., 1997; Wansard et al., 1999; Külköylüoğlu, 1999a, 1999b, 2009). These conditions are thought to have been stable for hundreds of thousands of years (Gooch and Glazier, 1991). Therefore, springs provide important knowledge about endemism, zoogeography, and pattern distribution in the past (e.g., post-Pleistocene colonization (Williams et al., 1990)). Organisms inhabiting springs should be adapted to these conditions. Shell chemistry may be used as a mirror of past changes in the water quality (Külcöylüoğlu, 1999a, 2005; Külcöylüoğlu and Yılmaz, 2006).

For shell chemistry studies to succeed, modern analogs are indispensable. Lack of such analogs prevents specialists from assessing the environmental conditions recorded by ostracod shell chemistry. As Holmes and Chivas (2002) demonstrated, trace element uptake is genus-specific; therefore, determining the environment-to-trace-element content in the shells requires establishing the mineral uptake by the species. By doing so, it would become possible to achieve quantitative reconstructions of past environments.

To study regional variations during global climate change, climatologists need a dense network of reliable paleoclimatic reconstructions over large regions. This requires integrating a vast database incorporating trace elements (Mg, Sr) and stable isotopes ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) from ostracod valves. By integrating trace element and stable isotope signatures, it may be possible to correct oxygen isotope composition for ostracod valves for the temperature effect and hence get a reliable record of the aquatic isotopic composition.

Based upon the importance of ostracods as paleoclimate indicators and their potential in springs, the aims of this study are to: 1) create modern analogs for two ostracod species (*Potamocypis similis* G. W. Müller, 1912 and *Psychrodromus olivaceus* (Brady & Norman, 1889)) associated with Karapınar Spring (Bolu, Turkey); 2) question the degree of correlation between the water Me/Ca ratio of Karapınar Spring and *P. similis* and *P. olivaceus* (where Me represents the Mg or Sr content in the ostracod valve); 3) compare the monthly Me/Ca ratio between these two species; 4) identify the stable isotope signatures left by

$\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ in *P. similis* and *P. olivaceus*; and 5) recognize the possible correlation between minor element ratios and isotopic ratios from the two species.

2. Materials and methods

2.1. Site description

Karapınar Spring (40°27'142"N, 30°50'162"E), at an elevation of 914 m a.s.l., is located about 1 km south of Lake Çubuk, Göynük, Bolu, Turkey (Figure 1). It is a rheocrene spring where water continuously flows through Göldere Creek, which originates from Lake Çubuk. Some amount of Karapınar spring water is brought to Göldere Creek by a pipe, where waters are mixed and flowing. There were no water plants covering the site.

2.2. Sampling and procedures

Materials were collected monthly from 24 October 2009 to 14 November 2010. Sampling was done using a plankton net (125- μm mesh size) from about 15 cm of depth within 1 m² of area. Physical and chemical parameters were measured monthly and recorded in situ (Table 1). The pH and redox potential values were measured with a Hanna model HI-98150 pH/ORP meter. Standard hydrogen electrode (SHE) was calculated from the field data of redox potential (mV). The other variables (dissolved oxygen (DO [mg L⁻¹]), oxygen saturation (Sat. %), electrical conductivity (EC [$\mu\text{S cm}^{-1}$]), water temperature (T(w) [°C]), and salinity (Sal [ppt]) were measured using a YSI-85 model oxygen-temperature meter. A geographical positioning system unit (GPS Garmin 45) was used to

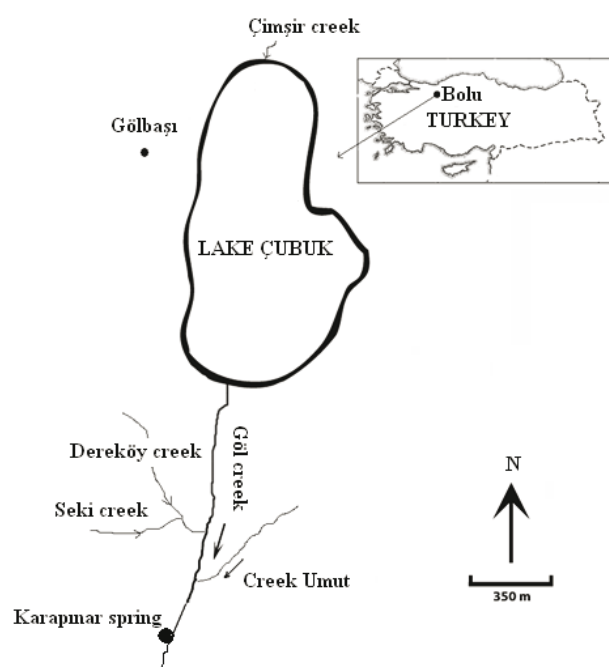


Figure 1. Location of Karapınar Spring.

Table 1. Sampling dates and 8 major environmental variables (pH, redox potential (SHE [mV]), electrical conductivity (EC [$\mu\text{S}/\text{cm}$]), dissolved oxygen (DO ([mg/L]), percent oxygen saturation (Sat.), salinity (Sal [ppt]), and air (Ta) and water temperature (Tw [°C])) measured at Karapınar Spring. Numbers of individuals collected of the two species, PS (*Potamocypis similis*) and PO (*Psychrodromus olivaceus*), are given, along with the mean and standard deviation (SD).

Date	pH	SHE	EC	DO	Sat	Sal.	Tw	Ta	PS	PO
24.10.2009	7.2	199.69	372	Na	Na	0.2	10.4	22.3	40	60
21.11.2009	7.52	199.52	190.2	Na	Na	0.1	10.2	11.2	43	38
19.12.2009	7.75	192.22	371.5	Na	Na	0.2	10.2	4.1	69	57
20.02.2010	7.36	197.58	361.2	Na	Na	0.2	9.8	14.6	23	15
20.03.2010	7.34	201.51	378.1	Na	Na	0.2	9.6	14.6	15	40
17.04.2010	7.11	216.02	356.3	Na	Na	0.2	10.2	20.6	13	17
22.05.2010	7.22	203.59	366.5	Na	Na	0.2	10.7	17.2	21	17
19.06.2010	7.24	201.78	385.3	Na	Na	0.2	10.1	24.8	47	21
24.07.2010	7.66	205.90	414	8.4	74.9	0.2	10.22	22.1	19	33
21.08.2010	7.27	209.29	366	9.6	84.4	0.18	10.24	24.1	54	63
18.09.2010	7.54	199.82	240	7.1	75.2	0.16	10.5	26.6	55	103
24.10.2010	7.38	208.05	345	9.7	86.5	0.17	10.3	12.4	59	37
14.11.2010	7.42	206.25	327	8.1	91.1	0.15	10	22.7	38	17
Mean	7.38	203.17	344.08	8.6	82.4	0.18	10.19	18.3	38.15	39.84
SD	0.19	6.02	61.58	1.1	7.15	0.03	0.28	6.55	18.45	25.53

record elevation (m) and coordinates. An anemometer was used to take ambient temperature.

About 200 mg of sediment materials collected from the sampling site was kept in 250-mL plastic containers and fixed in 70% ethanol. In the laboratory, materials were filtered using 3 standard sieves under tap water. Ostracods were separated from sediment under a stereomicroscope (Olympus BX51) and kept in 70% ethanol. The two species selected for this analysis differ in size. *P. similis* is a small ostracod (about 0.56 mm), whereas *P. olivaceus* is about 1.1 mm in length. Ten adult specimens of each of the two species were selected each month for shell chemistry analyses (stable isotopes and trace elements). Valves were separated and submerged in 10% hydrogen peroxide solution for about 10–15 min. Valves were then rinsed with distilled water 3 times. We used a fine brush (No. 000) to clean the valves in the H_2O_2 under a stereomicroscope. Each valve (left and right) was placed in a separate test tube and then onto a micropaleontological slide. The species were identified following Meisch (2000). Living specimens were used during the analyses.

2.3. Chemical analyses

Since there were no significant changes among the monthly values of the spring water (Table 1), 250 mL of spring

water was collected only on 21 September 2011. Water isotope analyses were conducted according to the procedure of analysis of trace and nontrace elements in waters and wastes by EPA Method 200.8 (inductively coupled plasma–mass spectrometry (ICP-MS)) at the Stable Isotope and Geochemistry Laboratory, Department of Environmental Engineering, Abant İzzet Baysal University, Bolu, Turkey.

Ostracod shell chemistry (trace elements and stable isotopes) of *Potamocypis similis* and *Psychrodromus olivaceus*, the two most common and abundant species in Karapınar Spring, was analyzed to determine the sources of water and the system's hydrological stability. Between 3 and 10 valves per monthly sample were thoroughly cleaned with MQ18 water and a fine brush (000) and then submitted for trace element (TE; left valves) and stable isotope (SI; right valves) analyses to the Department of Physics and Geology at California State University at Bakersfield (TE) and the Environmental Isotope Laboratory at the University of Arizona (SI), respectively.

Trace elements' intensity (Mg and Sr) and Ca were measured from clusters of 3 to 10 valves from the same month and analyzed for stable isotopes to generate a calibration signature of the Mg/Ca and Sr/Ca ratios for

both species, as well as to compare them with the $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ data. The percentage of each isotope was considered to be the same in the sample and the standard because the trace element isotopes are stable. Using minor isotopes minimizes interference problems with major isotopes like ^{40}Ca , which has the same mass as ^{40}Ar , the carrier gas for the plasma. The samples were dissolved in 5 mL of trace-grade HNO_3 and spiked with 25 μL of 1 ppm ^{59}Co solution as an internal standard. The spike resulted in a nominal 5 $\mu\text{g L}^{-1}$ of ^{59}Co . Using Elan 6100 ICP-MS, 3 replicates per sample were run with a precision of $\pm 0.04 \mu\text{g L}^{-1}$ for Mg, $\pm 1 \mu\text{g L}^{-1}$ for Ca, and $\pm 0.02 \text{ g L}^{-1}$ for Sr. Table 2 shows the Mg/Ca_v ($\mu\text{mol/L}$) and Sr/Ca_v ($\mu\text{mol/L}$) obtained from the trace element measurements, where the subscript 'v' indicates valve.

For stable isotopes ($\delta^{18}\text{O}$ and $\delta^{13}\text{C}$), 3 to 10 valves were grouped as a single sample to be analyzed for each month (Table 2). Oxygen and carbon-stable isotope ratios were measured with an automated carbonate-preparation device (Kiel III) coupled to a gas-ratio mass spectrometer (Finnigan MAT 252). Samples were reacted with dehydrated phosphoric acid under vacuum at 70 °C. The isotope ratio measurement was calibrated based on repeated measurements of NBS-19 and NBS-18; precision is $\pm 0.1\%$ for both $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ (one sigma). The values are expressed in delta per mil notation (δ , ‰), relative to the Vienna Pee Dee Belemnite standard (VPDB) (Table 2).

2.4. Statistical analyses

Ecological tolerance (Tol) and optimum (Opt) values for the two species were estimated using the C2 program for all variables of interest (Juggins, 2003). The weighted-averaging (WA) method was used to obtain the tolerance and optimum estimates. The species' optimum values were obtained as averages of all sampling sites (i.e. months), where the species' occurrence was weighted by the abundance in each month. Optimum values of individual species are assumed to correspond to the ecological tolerance of species to environmental variable(s); the species are expected to be abundant in sampling sites with values closer to their optimum values. Comparison of the water and valve chemistry was done using the Excel 97–2003 program at 0.05 significance level.

3. Results

The lower and upper ranges of the physicochemical values of the spring water did not show significant fluctuations. Accordingly, Karapınar Spring displayed characteristics of a cold (9.6–10.7 °C) and slightly alkaline (pH 7.11–7.75) freshwater (salinity 0.1–0.2 ppt) type of rheocrene spring (Table 1). During this study at Karapınar Spring, *Potamocypris similis* and *Psychrodromus olivaceus* were found year-round (Figure 2). The mean individual numbers of coexisting species populations did not show

significant differences ($P > 0.05$). The ecological response of both species was similar to pH, EC, T(w), and SHE (Table 3). Trace element and stable isotope data are compiled in Table 2.

Mg/Ca ratios were significantly different between the spring water and both of the species' valves (see Table 2). With the exception of an outlier recorded for *P. similis*, the Mg/Ca_v ($\mu\text{mol/L}$) (Figure 3) and Sr/Ca_v ($\mu\text{mol/L}$) (Figure 4) molar ratios of both species were relatively constant in value between October and March (Mg/Ca_v ($\mu\text{mol L}^{-1}$): 0.0059–0.0081; Sr/Ca_v ($\mu\text{mol L}^{-1}$): 0.00038–0.00042). The ratios were somewhat higher for *P. similis* than for *P. olivaceus*; however, they overlapped between May and September (Mg/Ca_v ($\mu\text{mol L}^{-1}$): about 0.0029–0.0038; Sr/Ca_v ($\mu\text{mol L}^{-1}$): 0.00030–0.00037). If one discards the outlier (Mg/Ca_v ($\mu\text{mol L}^{-1}$): 0.00115; Sr/Ca_v ($\mu\text{mol L}^{-1}$): 0.00054), there was a significant ($P < 0.05$) difference between the two species. By contrast, the Mg/Ca_v ($\mu\text{mol L}^{-1}$) ratios for *P. olivaceus* showed low values throughout the year (0.0026–0.0040), similar to the lower values recorded for *P. similis*. The Sr/Ca_v ($\mu\text{mol L}^{-1}$) ratios were equally low year-round (0.00028–0.00035). It is possible that the poor Ca content in the valves of *P. similis* and *P. olivaceus* may have resulted from analyzing preadult specimens. The strong correlation of trace element molar ratios between *P. similis* and *P. olivaceus* will be discussed in the next section.

The stable isotope record varied between *P. similis* and *P. olivaceus*. In *P. similis*, it was very patchy (see below). The isotopic values for both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ showed an overall variation as large as 1.5‰ VPDB. For example, Figure 5A shows that $\delta^{18}\text{O}$ for *P. similis* ranged from -7.28% to -7.73% VPDB, and $\delta^{13}\text{C}$ oscillated between -7.92% and -8.73% VPDB. *P. olivaceus* also showed little variation in isotopic values. $\delta^{18}\text{O}$ fluctuated from -7.70% to -7.99% VPDB, whereas $\delta^{13}\text{C}$ ranged between -7.94% and -9.00% VPDB (Figure 5B).

4. Discussion

In this section, we discuss the implications of the trace element and stable isotope signatures obtained from *P. similis* and *P. olivaceus* from Karapınar Spring. Were we able to establish a modern analog for paleoclimate studies in the region using *P. similis* and/or *P. olivaceus*? How reliable are the patterns shown by these two species? Is there a correlation or comparison between the Me/Ca_v ($\text{Me} = \text{Mg}$ or Sr) molar ratios and the isotopic values?

When examining environmental controls on ostracod valve chemistry, it is important to consider the influence that temperature, $\text{Me}/\text{Ca}_{\text{water}}$, $[\text{Me}]_{\text{water}}$, and salinity have in shell formation. However, biology and taxonomy also play a role in the relationship of shell chemistry to the local environment. In nonmarine environments,

Table 2. Karapınar Spring Me/Ca_w ($\mu\text{mol/L}$) and Me/Ca_v ($\mu\text{mol/L}$) ratios of the right valves for *Potamocypis similis* (PS) and *Psychrodromus olivaceus* (PO). *: Low voltage signal. Unreliable data. Note the similar values of water chemistry data due to one-time analyses.

Date	Valve chemistry										Molar ratio							Water chemistry				
	Mg	Ca	Sr	Co	Mg/Ca	Sr/Ca	Co/Mg	Co/Ca	Mg	Ca	Sr	Ca	Mg	Ca	Sr	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	Mg (w)	Ca (w)	Mg/Ca (w)	T °C (w)	
24.10.2009	19.03	9182.4	6.68	5.18	0.0021	0.0007	0.272	0.0006	0.7826	229.56	0.0762	-7.95	-7.82	631.8	64314	0.00982	10.4					
21.11.2009	28.16	15620	11.5	5.26	0.0018	0.0007	0.187	0.0003	1.1583	390.51	0.1309	-8	-7.79	631.8	64314	0.00982	10.2					
19.12.2009	26.37	13725	10.5	5.29	0.0019	0.0008	0.2	0.0004	1.0847	343.13	0.12	-7.94	-7.72	631.8	64314	0.00982	10.2					
20.02.2010	25.84	13647	9.64	5.32	0.0019	0.0007	0.206	0.0004	1.0628	341.17	0.11	-8.49	-7.99	631.8	64314	0.00982	9.8					
20.03.2010	26.58	14149	9.75	5.27	0.0019	0.0007	0.198	0.0004	1.0935	353.73	0.1113	-8.45	-7.85	631.8	64314	0.00982	9.6					
17.04.2010	23.53	14006	8.65	5.23	0.0017	0.0006	0.222	0.0004	0.9678	350.14	0.0988	-8.68	-7.7	631.8	64314	0.00982	10.2					
22.05.2010	26.77	13688	9.19	5.15	0.002	0.0007	0.193	0.0004	1.101	342.21	0.1049	-8.73	-7.83	631.8	64314	0.00982	10.7					
19.06.2010	12.24	5095.5	3.15	5.18	0.0024	0.0006	0.423	0.001	0.5036	127.39	0.0359	-9	-7.72	631.8	64314	0.00982	10.1					
24.07.2010	22.84	14342	8.83	5.03	0.0016	0.0006	0.22	0.0004	0.9393	358.54	0.1007	-8.67	-7.78	631.8	64314	0.00982	10.22					
21.08.2010	15.64	9126.7	6.85	5.09	0.0017	0.0008	0.326	0.0006	0.6433	228.17	0.0782	-8.14	-7.81	631.8	64314	0.00982	10.24					
18.09.2010	5800	55485	391	6.95	0.1045	0.0071	0.001	0.0001	238.58	1387.1	4.4668	-8.94	-7.72	631.8	64314	0.00982	10.5					
24.10.2010	27.84	13230	9.61	5.1	0.0021	0.0007	0.183	0.0004	1.1452	330.76	0.1097	-7.99	-7.79	631.8	64314	0.00982	10.3					
14.11.2010	23.3	12892	9.72	5.24	0.0018	0.0008	0.225	0.0004	0.9583	322.3	0.111	-7.99	-7.79	631.8	64314	0.00982	10					
Date	Mg	Ca	Sr	Co	Mg/Ca	Sr/Ca	Co/Mg	Co/Ca	Mg	Ca	Sr	$\delta^{13}\text{C}$	$\delta^{18}\text{O}$	Mg (w)	Ca (w)	Mg/Ca (w)	T °C (w)					
24.10.2009	11.35	1623	1.93	4.77	0.007	0.0012	0.42	0.0029	0.4668	40.576	0.0221	-7.93	-7.73	631.8	64314	0.00982	10.4					
21.11.2009	6.582	1844.3	1.6	4.68	0.0036	0.0009	0.711	0.0025	0.2708	46.107	0.0183	*-7.65	*-7.55	631.8	64314	0.00982	10.2					
19.12.2009	3.586	959.66	0.81	4.59	0.0037	0.0008	1.28	0.0048	0.1475	23.992	0.0093	*8-17	*-7.78	631.8	64314	0.00982	10.2					
20.02.2010	4.802	1339.2	1.12	4.68	0.0036	0.0008	0.976	0.0035	0.1975	33.481	0.0128			631.8	64314	0.00982	9.8					
20.03.2010	4.753	963.58	0.88	4.59	0.0049	0.0009	0.965	0.0048	0.1955	24.089	0.01	*-7.81	*-7.69	631.8	64314	0.00982	9.6					
17.04.2010	4.131	1019.1	0.84	4.68	0.0041	0.0008	1.134	0.0046	0.1699	25.478	0.0096			631.8	64314	0.00982	10.2					
22.05.2010	2.652	197.95	0.29	4.71	0.0134	0.0015	1.775	0.0238	0.1091	4.9488	0.0033			631.8	64314	0.00982	10.7					
19.06.2010	7.561	3377.7	2.41	4.72	0.0022	0.0007	0.625	0.0014	0.311	84.442	0.0275	*-7.63	*-6.99	631.8	64314	0.00982	10.1					
24.07.2010	3.91	1819.5	1.28	4.69	0.0021	0.0007	1.199	0.0026	0.1608	45.488	0.0146	-8.72	-7.43	631.8	64314	0.00982	10.22					
21.08.2010				4.34								-7.92	-7.28	631.8	64314	0.00982	10.24					
18.09.2010	5.729	3221.2	2.13	4.59	0.0018	0.0007	0.801	0.0014	0.2357	80.529	0.0243			631.8	64314	0.00982	10.5					
24.10.2010	4.807	2613.9	2.08	4.65	0.0018	0.0008	0.968	0.0018	0.1978	65.347	0.0238	-8.73	-7.45	631.8	64314	0.00982	10.3					
14.11.2010	5.499	2353	1.93	4.62	0.0023	0.0008	0.841	0.002	0.2262	58.824	0.022	-7.89	-7.68	631.8	64314	0.00982	10					

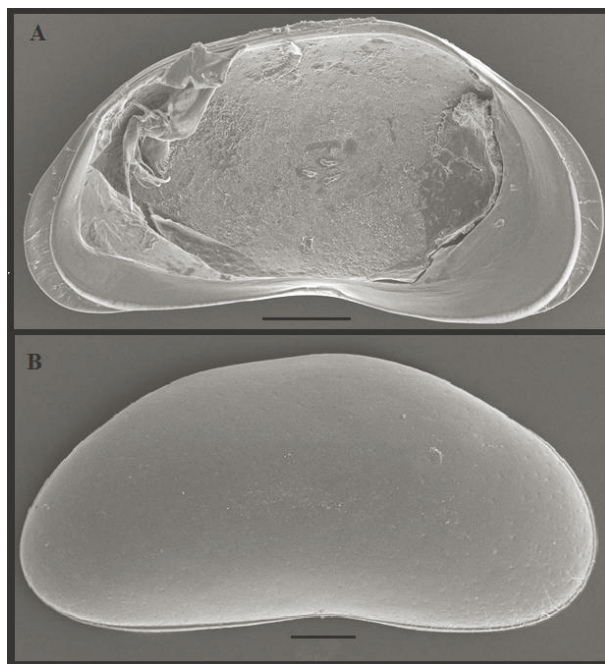


Figure 2. SEM photos of (A) *Potamocypris similis*, left valve inner view; and (B) *Psychrodromus olivaceus*, carapace right valve external view. Scale bar = 100 µm.

Table 3. Optimum and tolerance values of *Potamocypris similis* and *Psychrodromus olivaceus* to pH, EC (electrical conductivity), T(w) (water temperature), T(a) (air temperature), and SHE (standard hydrogen electrode: redox potential).

Species	Count	Max	N2	pH		EC		Tw		Ta		SHE	
				Opt	Tol	Opt	Tol	Opt	Tol	Opt	Tol	Opt	Tol
<i>P. similis</i>	13	69	10.7	7.42	0.19	336.1	65.21	10.23	0.23	17.71	7.87	202.2	6.04
<i>P. olivaceus</i>	13	103	9.43	7.42	0.2	330.9	70.62	10.24	0.27	18.91	7.67	202.1	5.82

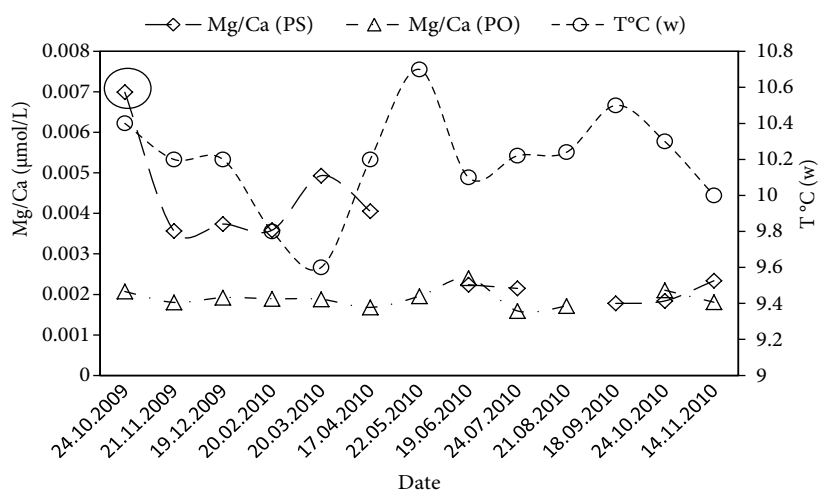


Figure 3. Mg/Ca, (µmol/L) ratios for *P. similis* (PS) and *P. olivaceus* (PO) compared to Karapınar Spring water temperature (T °C (w)). Apparently there is no correspondence between molar ratios from either species with water temperature. Note that the far left point in the circle is an outlier, possibly because of a contaminated shell.

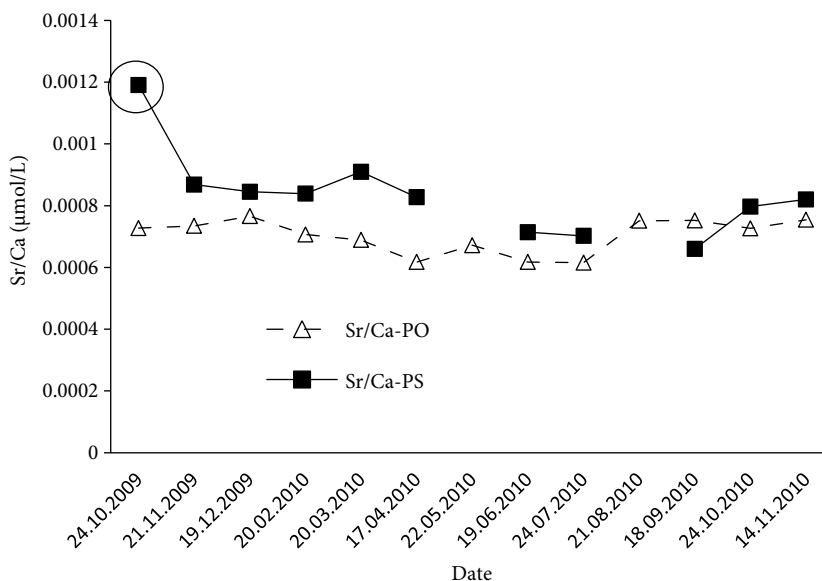


Figure 4. Sr/Ca ratio in the valves of *P. similis* (PS) and *P. olivaceus* (PO) from Karapınar Spring. Note that the far left point in the circle is an outlier.

this relationship appears to be more complex than in the oceanic realm due to high environmental variability and fast calcification processes in response to changes in these variables. Ostracod life-cycles show a wide range of variability, as well. Some species reach maturity in as little as 28 days (e.g., *Limnocythere staplini*, *Cypridopsis vidua*), while others may take up to 2 years (e.g., *Darwinula stevensoni*), strongly influenced by water permanence in addition to the 4 variables listed earlier (Palacios-Fest, 1994; Anderson et al., 1998). Therefore, collecting live organisms from natural habitats does not necessarily mean that the valves were calcified under the conditions measured at the time of sampling, unless one is working with a very short-lived species.

Both species studied were found monthly during our study. It is known that *P. olivaceus* inhabits spring habitats and/or water bodies connected to springs (e.g., creeks or small ponds fed by spring waters). Although males are rare, adult females and juveniles of *P. olivaceus* can be found throughout the year. Based on Meisch (2000), we assume that *P. olivaceus* has an approximately 6-month life-cycle, while *P. similis* may be a short-lived species. *P. olivaceus* shows at least two annual generations between April–May and August–September (Meisch, 2000). According to Külköylüoğlu et al. (2012), there was a significant correlation between water temperature and occurrence of *P. olivaceus* ($r = -0.257$, $P < 0.05$) in different water bodies of Kahramanmaraş (Turkey). Palacios-Fest and Dettman (2001) and Dettman et al. (2002) demonstrated the strong correlation between temperature and Mg/Ca_{valve} in studies conducted in northern Mexico, and Wansard and

Mezquita (2001) did likewise in Spain. Our observations from Karapınar Spring correspond with the earlier studies mentioned above and extend ecological knowledge about the species.

Unlike *P. olivaceus*, we have poor information about *P. similis*. For about the last decade (after Meisch, 2000), it has been reported, along with some additional ecological data, that *P. similis* is indeed a rare species. Meisch (2000) stated that he found the species from July to August. Similarly, it was reported from creeks, streams, and shallow littoral zones of lakes between July and August in Diyarbakır and Erzincan (Turkey) during the year 2006 (Akdemir, 2009). However, most recently, the species has been found in different months in Turkey (Sarı and Külköylüoğlu, 2010) and in Italy (Pieri et al., 2009). Although a positive correlation was shown with the dissolved oxygen in cool waters (ca. 15 °C) (Külköylüoğlu and Sarı, 2012), we still have poor ecological knowledge about the species. During the present study, the species was found year-round in relatively constant pH, EC, temperature, and salinity values (Table 1). Compared to *P. olivaceus*, *P. similis* tolerance ranges to environmental variables were not statistically significant ($P > 0.05$) (Table 2). However, the two species show distinct mineral uptake patterns. On the one hand, the relatively unvarying Me/Ca ratios (for both Mg and Sr) displayed by *P. olivaceus* seem to be influenced by its crenophilic preference, i.e. driven by groundwater. On the other hand, *P. similis* exhibits what may be interpreted as a biseasonal pattern with higher Me/Ca ratios between October and March and lower values from May to September; but does that imply that it is climate-driven?

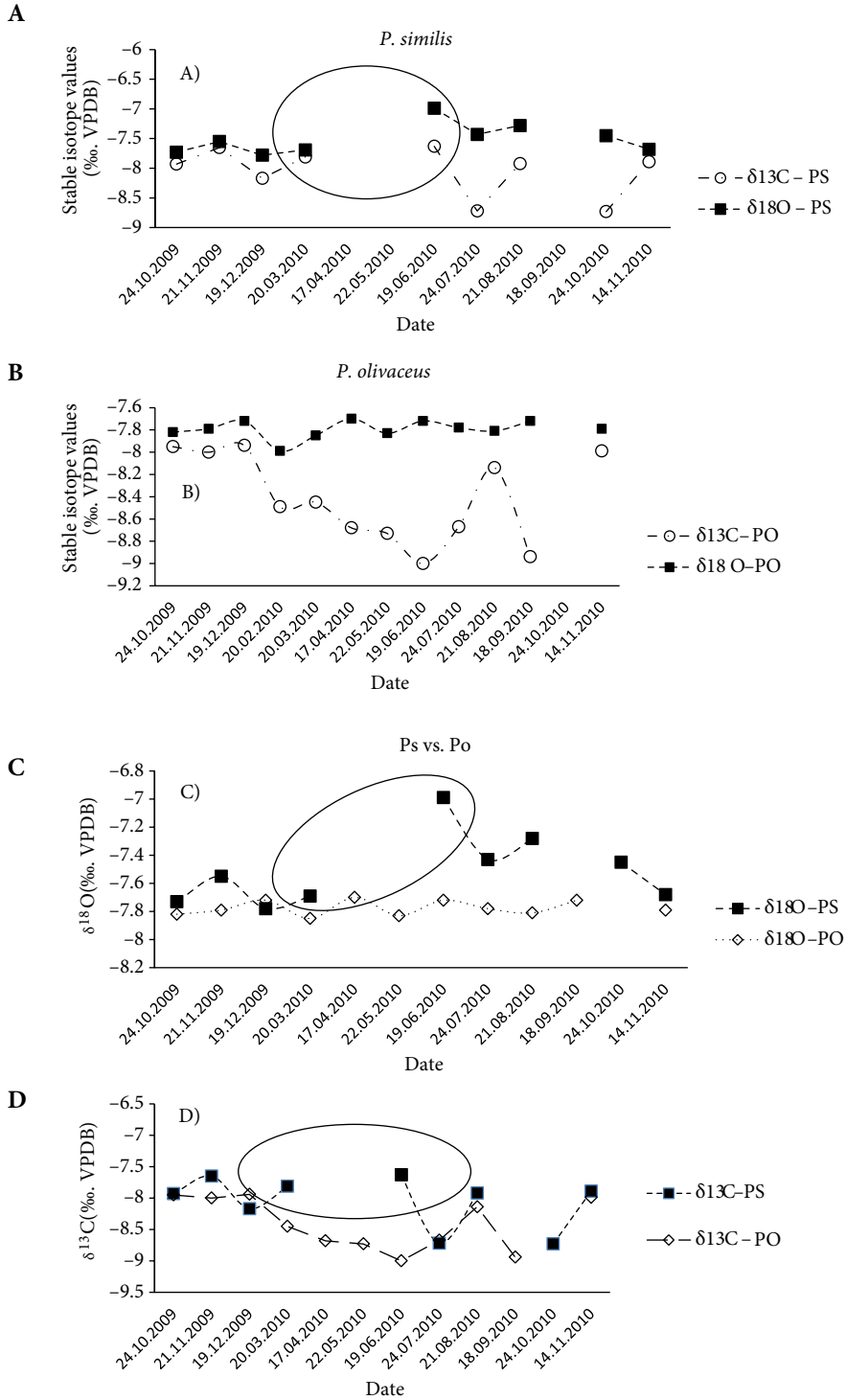


Figure 5. Stable isotope signatures obtained from two ostracod species from Karapınar Spring (Bolu, Turkey) collected from 24 October 2009 through 14 November, 2010. δ¹⁸O (closed squares) and δ¹³C (open circles) for (A) *Potamocypris similis*, (B) *Psychrodromus olivaceus*, (C) δ¹⁸O *P. similis* (open diamonds) vs. *P. olivaceus* (closed triangles), and (D) δ¹³C *P. similis* vs. *P. olivaceus*. Ellipses represent the missing values.

Valve chemistry of both species (*P. olivaceus*, *P. similis*) was compared in Karapınar Spring (Figure 3). Accordingly, the ratio of Mg/Ca ($\mu\text{mol/L}$) was significantly different between the spring water and the species' valves (keeping in mind that there was one-time sampling from the spring). The ratio was also significantly ($P < 0.05$) different between the valves of the two species. The Mg/Ca_v ($\mu\text{mol/L}$) of *P. olivaceus* was relatively constant within the study period; however, the Mg/Ca_v ($\mu\text{mol/L}$) in the valves of *P. similis* showed some variability that may be attributed to seasonal variations or the ostracod population. It seems that the water temperature did not correlate to the Mg/Ca_v ($\mu\text{mol/L}$) ratio in the valves of these species (Figure 3).

Studying *Limnocythere inopinata* from a brackish water lake in Mongolia, Van der Meeren et al. (2011) showed that the Sr/Ca ratio of the valves correlated better to Sr/Ca_{water} than Mg/Ca_{valve} did to Mg/Ca_{water}. If this is true, then it may be inferred that Sr/Ca is less affected by calcification rate and temperature (Van der Meeren et al., 2011). However, because we did not have monthly analyses of water chemistry throughout the year, it is suggested that water chemistry should also be analyzed on a monthly basis, especially if moving away from the spring.

Figure 3 shows the Mg/Ca_v ($\mu\text{mol/L}$) ratios against water temperature between October 2009 and November 2010. It is clear that neither species appears to respond to water temperature as we expected. This study adds to some previous investigations that have proven that it is difficult to use ostracod shell chemistry as a tool for paleoclimate reconstructions (Decrouy et al., 2012).

The search for simple controlling factors may also pose an obstacle for understanding metal uptake by ostracods. Two different species from the same environment prove that there is not a straightforward mechanism of shell calcification. This is evident in the case of *P. similis* where, at least once, the shells showed that poorly calcified organisms produced elevated Me/Ca_{valve}. This problem was first identified by Cadot and Kaesler (1977).

The stable isotope record for *P. similis* was very patchy, along with the small range of values displaying statistically high covariance (between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$) throughout the year; however, lack of reliable data between November 2009 and June 2010 makes the record difficult to interpret (Figure 5A). Four samples (November and December 2009, and March and June 2010) yielded unreliable values due to low voltage (encircled values). No data were obtained for April, May, or September 2010, creating a serious information gap. The data, however, were included in this study because they appear to fall within the range of other accepted values. The scant data show that the $\delta^{13}\text{C}$ values range between -7.92‰ and -8.73‰ VPDB with an undefined pattern but little variability. Similarly, the $\delta^{18}\text{O}$ values oscillate between -7.28‰ and -7.73‰

VPDB, a 0.45‰ variability, which is negligible. The $\delta^{13}\text{C}$ trend follows that of $\delta^{18}\text{O}$ throughout the year, implying that the covariant pattern may respond to increasing/decreasing trends in seasonal water temperature and local precipitation. The question is if this patchy trend may be related to seasonality. New data will be required to respond to this question. *P. olivaceus* shows a pattern in which the two isotopic signatures gradually diverge throughout the year with no statistical correlation (Figure 5B). $\delta^{18}\text{O}$ values are in general stable around -7.70‰ and -7.99‰ VPDB. It may be inferred that the oxygen isotope uptake is that from groundwater. By contrast, the $\delta^{13}\text{C}$ values show gradually lighter values (-7.82‰ to -9.00‰ , VPDB) between October 2009 and June 2010 with an abrupt return to heavier values in August (-8.14‰ , VPDB), only to drop back to lighter values in September (-8.94‰ VPDB) and finally return to -7.99‰ VPDB, similar to the range recorded a year earlier. The lack of correlation between $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ may also imply the influence of groundwater mixing with surface water in this crenophilic species.

Comparing the oxygen isotopic values between *P. similis* and *P. olivaceus*, it is clear that the species respond somewhat differently, regardless of the scant data available for *P. similis* and the overlap recorded between April and August. The pattern may be similar to that shown by trace elements discussed previously. Figure 5C shows that *P. similis* reacts much more strongly to seasonality, whereas *P. olivaceus* responds to groundwater. By contrast, $\delta^{13}\text{C}$ values for *P. similis* and *P. olivaceus* overlap throughout the year, displaying a similar trend (Figure 5D).

The results of this study call into question the validity of ostracod Mg/Ca and Sr/Ca molar ratios as reliable tools for the paleoenvironmental reconstruction of ancient lakes. More precisely, is it possible to identify the environmental conditions that affect mineral uptake in the two species that are the subjects of this investigation? This question will remain unanswered until a more detailed monthly analysis includes collecting water data every time. Any attempt to extrapolate Karapınar Spring Me/Ca molar ratio values to fossil ostracods is undesirable at this point. Stable isotopes seem to generate better evidence of environmental impact in ostracod shell chemistry, as *P. similis* seems, in spite of its fragmented record, to respond to seasonal variations not shown by *P. olivaceus*. To verify the significance of stable isotopes as proxy tools will require closing the sampling and analytical gaps obtained during this research.

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