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RESEARCH PAPER

On the Relationship of Ostracod Species (Crustacea) to Shallow Water Ion and Sediment Phosphate Concentration Across Different Elevational Range (Sinop, Turkey)

Mehmet Yavuzatmaca[1,]*, Okan Kulkoyluoglu[1], Ozan Yilmaz[1], Derya Akdemir[2]

[1] Abant İzzet Baysal University, Faculty of Arts and Science, Bolu, Turkey.
[2] Marmara University, Faculty of Arts and Science, İstanbul, Turkey.

* Corresponding Author: Tel.: +90.374 2541000/1348; Fax: ;
E-mail: yavuzatmaca_m@ibu.edu.tr

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Abstract

In order to investigate the relationship of freshwater ostracods with some of those physico-chemicals and sediment phosphate concentrations, 97 shallow aquatic bodies were sampled during July 2014 in the Sinop province (Turkey). A total of 3083 individuals belonging to 28 ostracod taxa are reported from 76 of 97 sites. While 24 of these taxa were firstly reported from the province, *Bradleytriebella tuberculata* is a newly reported species for Turkish Ostracoda fauna. Species diversity (H'=1.87) increased around the middle elevations (601-750 m) whereas it was decreased in low and high elevations. Results of ANOSIM analyses exhibited no significant difference among species composition of eight groups with 150 m elevational range (P>0.05). Cosmoecious species occurred in almost all elevational ranges whereas others seemed to prefer low to middle elevational ranges. A significant effect of water temperature, magnesium content of water, and total organic phosphate of sediments (P<0.05) were observed on ostracod assemblages. It appears that rather than number of sampling sites, habitat quality and spatial differences played critical roles on the species diversity.

Keywords: Ostracoda, cosmoecious species, diversity, phosphate, bioindicator.

Introduction

Until an equilibrium is reached, exchange of gases and ions among air, water and sediments are known to continue (Boyd, 2000). Of which, sediments act as a sink or source for a variety of chemical and pollutants in aquatic systems (Adams, Kimerle, & Barnett, 1992). The contaminants enter the water body via the disposal of liquid effluents, runoff and leachates carrying chemicals from urban, industrial and agricultural activities and from atmospheric deposition (Mucha, Vasconcelos, & Bordalo, 2003). For example, the key component of fertilizer "phosphorous (P)" is generally bound in sediment in various forms with iron, aluminium and as calcium phosphates (Boyd, 2000), and then it is transferred from sediment to water via mobilization (physical, chemical and biological) and transport processes (Boström, Andersen, Fleischer, & Jansson, 1988). Besides, ionic composition (anion and cations) of surface waters is also controlled by rock dominance, atmospheric precipitation, and evaporation-precipitation process (Wetzel, 2001). Hence, physico-chemical characteristics of waters to which organisms depend on can be affected by such changes (Boyd, 2000). However, most organisms show different levels of responses to such changes. Species with high tolerance levels can tolerate and survive in such habitats but others with no or low tolerances either migrate to nearby habitats or eliminated. If we know or estimate response levels of particular (aquatic) organisms to such changes, those organisms can be used as bio-indicator of water quality (Friedrich, Chapman, & Beim, 1996).

Ostracods are small bivalved aquatic invertebrates (0.3-5 mm long) (Meisch, 2000). They are very common in a variety of marine and non-marine environments such as deep bottom of seas, lagoons, estuaries, lakes, ponds, thermal waters, creeks, and troughs (Delorme, 1991; Külköylüoğlu, 2013), and they can be swimmer or non-swimmer benthic and planktonic in these habitats (Delorme, 1991). Also, they show species specific response and tolerances to different physico-chemical variables of aquatic bodies (Benson, 1990). Recent review of Ruiz *et al.* (2013) stated the importance of using freshwater ostracods as indicative of different environmental conditions (e.g., anthropogenic impacts, toxicity tests). Besides, ostracods are important members of benthic food web since they have similar food sources (e.g., algae, organic detritus, dead and living plant parts, body of dead animals) with amphipods and oligochaetes but are also the prey of other species (e.g., fish) (Modig, Van de Bund, & Olafsson, 2000; Karanovic, 2012). Additionally, benthic organisms consume and bio-accumulate the chemicals bonded to sediment (may be harmful) in their body that allow the transfer of chemicals from sediment to the higher organisms (Adams *et al.*, 1992). This reveals the importance of using both sediment and benthic organisms for water quality assessments (Mucha *et al.*, 2003). As can be understood from the information described above, ostracods can be one of the best organisms for water quality and biological monitoring studies.

Up to the present study, there has been no comprehensive and extensive field study dealing with freshwater ostracods in the Sinop province. The species reported (see Discussion) in the literature from the province are the outcome of samples collected by chance (Ghetti, 1973; Kaleli, 1993; Kılıç, 2001). Therefore, the present study is the first comprehensive field study in the Sinop province dealing with distribution and ecology of freshwater ostracods. Thus, the goals of the present study were i) to investigate the relationships of ostracod species with different physico-chemical, ion and sediment phosphate concentration of shallow waters in Sinop, ii) to determine species distribution and dis/similarities of species composition between different elevational ranges, and iii) to assess which ecological variables best explain the local ostracod species composition.

Materials and Methods

Study Site

The province of Sinop (5862 km^2 of surface area) has been constructed on the Boztepe Cape and Peninsula which is the most northern geographical point located on the north coast of the Black Sea (Turkey) between 41°12′ - 42°06′ N and 34°14′ - 35°26′ E. In the region, mountains are stretched parallel to the Black Sea. As a result, Sinop province has a humid and semi-humid climate where winter is cool and summer is warm. The seasonal variation in temperature throughout the province is limited and thus mean temperature of the coastal and inland towns are 13-15 and 12-14°C, respectively (Sinop Valiliği, 2016).

Sampling and Measurements

A total of 97 sampling sites with 11 different aquatic habitats (trough, creek, water body, pool, pond, reservoir, rice paddy, spring, stream, water fall and wetland) was randomly visited and sampled between 12-15 July, 2014 (Figure 1). All environmental variables and basic geographical data were recorded before sampling *in situ* to prevent water disturbance. Dissolved oxygen concentration (DO, mg L^{-1}), percent oxygen saturation (%sat.), water temperature (Tw, °C), electrical conductivity (EC, µS cm^{-1}), pH, atmospheric pressure (mmHg), oxidation-reduction potential (ORP, mV) were recorded by using a YSI-Professional Plus. A Kestrel-3000 model anemometer was used to obtain air temperature (Ta, °C), wind speed (m s^{-1}), wind chill (w. chill, °C), heat index (HI, °C), dew point (Dp, °C) and relative humidity (%) while geographical data (elevation, coordinates) were recorded with a geographical positioning system (GARMIN etrex Vista H GPS) (Table 1).

Then after, 100 ml of water for cation and anion (sodium (Na^{+1}), ammonium (NH_4^{+1}), potassium (K^{+1}), magnesium (Mg^{+2}), lithium (Li^{+1}), calcium (Ca^{+2}), fluoride (F^{-1}), chloride (Cl^{-1}), nitrite (NO_2^{-1}), bromide

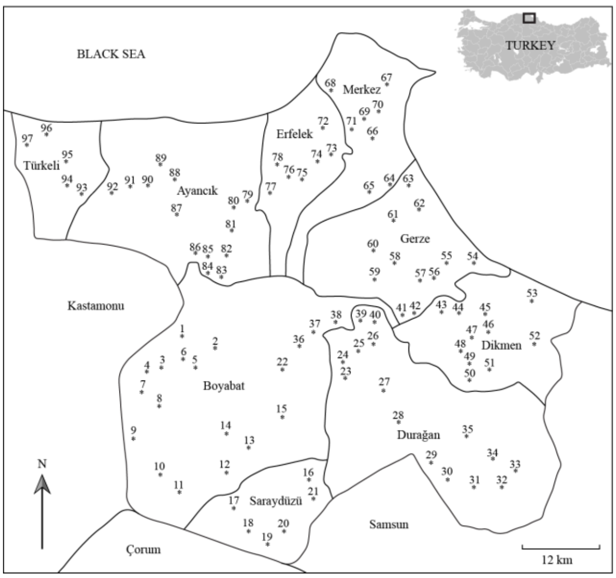

Figure 1: Sampling sites (97) from nine counties (Türkeli, Ayancık, Erfelek, Merkez, Gerze, Dikmen, Durağan, Saraydüzü and Boyabat) of the Sinop province.

Table 1. Minimum, maximum, average and standard deviation of environmental variables were recorded in the Sinop province. bcl means below critical level.

Variables	Min	Max	Mean	Standard Deviation
pH	7.17	9.28	8.02	0.40
Dissolved oxygen (mg L^{-1})	0.30	16.22	3.77	3.69
%dissolved oxygen (%sat.)	0.24	204.20	41.83	41.74
Electrical conductivity (µS cm^{-1})	102.50	13938.00	769.88	1443.65
Water temperature (ºC)	10.40	35.50	21.70	5.60
Air temperature (ºC)	15.40	35.40	27.28	4.59
Atmospheric Pressure (mmHg)	661.10	758.50	709.29	28.81
Oxidation and reduction potential (mV)	-43.30	194.80	142.10	34.56
Relative Humidity (%)	30.70	94.90	53.96	13.23
Wind (m s^{-1})	0.00	4.10	0.79	0.74
Wind Chill (ºC)	16.50	35.50	27.38	4.46
Heat index (ºC)	16.30	40.60	28.68	5.59
Dew point (ºC)	9.60	24.40	16.62	2.95
Elevation (m)	-3.00	1152.00	582.46	347.48
Sodium (mg L^{-1})	1.04	476.45	26.65	58.09
Ammonium (mg L^{-1})	0.27	0.43	0.35	0.11
Potassium (mg L^{-1})	0.18	72.15	2.51	7.49
Magnesium (mg L^{-1})	0.75	78.78	15.25	15.20
Lithium (mg L^{-1})	0.001	0.02	0.01	0.01
Calcium (mg L^{-1})	6.07	559.98	71.26	59.38
Fluoride (mg L^{-1})	0.02	2.08	0.13	0.24
Chloride (mg L^{-1})	1.05	366.16	13.93	41.47
Nitrite (mg L^{-1})	bcl	bcl	bcl	bcl
Bromide (mg L^{-1})	bcl	bcl	bcl	bcl
Nitrate (mg L^{-1})	0.15	39.06	4.33	7.87
Phosphate (mg L^{-1})	bcl	bcl	bcl	bcl
Sulphate (mg L^{-1})	1.12	2908.92	110.54	363.76
Total Phosphate (mg kg^{-1})	0.06	2.28	0.38	0.27
Inorganic Phosphate (mg kg^{-1})	0.005	3.06	0.51	0.72
Organic Phosphate (mg kg^{-1})	0.01	0.39	0.10	0.08

(Br^{-1}), nitrate (NO_3^{-2}), phosphate (PO_4^{-3}) and sulphate (SO_4^{-2}) analyses was collected from each sampling site in plastic bottles and preserved in about 4ºC of container. Similar to water samples, sediment from each sampling site was gathered in Eppendorf to analyse amount of in/organic phosphate (phosphorous) and total phosphate (mg kg^{-1}). Ostracod samples were compiled with a standard sized hand net (200 µm in mesh size) from ca 4 cm over sediment and fixed with 70% of ethanol in 250 ml plastic bottles.

In the laboratory, water samples were firstly filtered through syringe-type membrane filter (0.45 µm) for cation and anions analyses that are performed with the directions in the standard method no: 4110 using Ion Chromatography (Dionex 1100) in Department of Environmental Engineering, Abant İzzet Baysal University (Bolu, Turkey). Dionex IonPac AS9-HC anion column (4x250 mm), Dionex IonPac AG9-SC (4x50 mm) pre-column and Dionex ASRS-300 4 mm suppressor were used for anion analyses along with the application of 9 mm Na_2CO_3 solution as a carrier phase at a flow rate of 1.0 ml min^{-1}. In cation analyses, Dionex IonPac CS12A cation column, Dionex IonPac CG12A (4x50 mm) pre-column and Dionex CSRS-300 4 mm suppressor were used. 10 ml of 1M meta sulfonic acid (MSA) were received as a carrier phase to prepare 1 litre solution. Flow rate of prepared solution was 1.0 ml $min.^{-1}$ during analysis. Sediment samples were firstly dried in drying oven at 40ºC (at least 24 hours), and then sequential extraction procedure provided in Ruban *et al.* (1999) was used for determination of phosphorous forms (inorganic, organic and total) in sediment.

Each ostracod sample was filtered through four standardized sieves (0.5, 1.0, 1.5 and 2.0 mm mesh size) under tap water and then specimens were sorted from sediments under a stereomicroscope (Olympus ACH 1X). Subsequently, we dissected their soft body parts in lacto-phenol solution for taxonomic identification. A light microscope (Olympus BX-51) was used for taxonomic identification. We used taxonomic keys of Meisch (2000) and Karanovic (2012) for species identification. Valves of some species were kept in micropaleontological slides for Scanning Electron Microscope (JEOL JSM-6390LV SEM) photography at Abant İzzet Baysal University. All of the ostracod samples were kept at the Limnology Laboratory of Abant İzzet Baysal University, Bolu, Turkey and are available upon request.

Statistical Analyses

We determined eight groups (ranges) with 150 m of elevational intervals from 0 to 1152 m a.s.l. Analysis of similarity (ANOSIM) was conducted to compare dis/similarities in ostracod communities

Table 2. Shannon-Wiener Index values (H', Variance H and Exp. H), habitat types, abundance and percent abundance (%abund.) of 28 taxa in eight groups 150 m elevational intervals in the Sinop province. *Candonopsis* sp. and *Candona* sp. were classified at genus level due to damaged soft body parts and the presence of only juveniles, respectively. Underlined bold numbers on the most right side show number of sampled sites in the concerned elevational intervals

Shannon-Wiener Index				Ostracoda																												
H'	Variance H	Exp. H	HABITATS	*Cyprideis torosa*	*Candona angulata*	*Candona neglecta*	*Fabaeformiscandona* cf. *latens*	*Fabaformiscandona balatonica*	*Pseudocandona albicans*	*Ilyocypris bradyi*	*Ilyocypris brehmi*	*Ilyocypris inermis*	*Ilyocypris gibba*	*Cypria optalmica*	*Physocypria kraepellini*	*Cypridopsis vidua*	*Potamocypris similis*	*Potamocypris fulva*	*Potamocypris pallida*	*Heterocypris incongruens*	*Heterocypris salina*	*Heterocypris reptans*	*Psychrodromus olivaceus*	*Psychrodromus fontinalis*	*Herpetocypris chevreuxi*	*Stenocypris bolieki*	*Bradleytriebella tuberculata*	*Cypretta seurati*	*Limnocythere inopinata*	*Candonopsis* sp.	*Candona* sp.	Intervals
1.02	0.00	2.78	Creek							10		2								1												1200 m
			Pool							1																						
			Pond																													
			Spring									18																				**14**
			Trough					1		200										56			25	1							1	
			Water body							50																					12	
			%abund.					0.03		8.47		0.65								1.85			0.81	0.03							0.42	1050 m
1.73	0.00	5.64	Reservoir																													
			Pond							4				58																		**10**
			Trough							60						7				3	62		23	17							9	
			%abund.							2.08				1.88		0.23				0.1	2.01		0.75	0.55							0.29	900 m
1.74	0.00	5.70	Creek									10																			1	
			Pool									39						1													3	
			Pond												4																	
			Stream																													**12**
			Trough							2										5	39		21									
			Water body							46																						
			Water fall							22								64					3									
			Wetland							8								24						1						3	1	
			%abund.							2.53		1.59			0.13			2.89		0.16	1.27		0.78	0.03						0.1	0.16	750 m
1.87	0.00	6.50	Creek		7					11							2															
			Reservoir																													
			Spring			12				82		28					7	19						2								**9**
			Trough		5		5			19											2		52		13						5	
			Wetland							1										1												
			%abund.		0.39	0.39	0.16			3.67		0.91					0.29	0.62		0.03	0.06		1.69	0.06	0.42						0.16	600 m
1.53	0.00	4.62	Creek							10	6							3		2												
			Reservoir													6																**12**
			Stream																3													
			Trough		1					10	2									86	67		1	2								
			%abund.		0.03					0.42	0.26					0.19		0.1	0.1	2.85	2.17		0.03	0.71								450 m
1.75	0.00	5.75	Creek							215											2		1						1			
			Rice paddy								29									9						58		5				**19**
			Spring																	6												
			Stream									1		6						1			1									
			Trough						3	133		4	1					2		148	59		242								8	
			Water body																				2									
			Waterfall							25								1			7		10								60	
			%abund.						0.1	12.1	0.94	0.16	0.03	0.19				0.1		5.32	2.21		8.3			1.88		0.16	0.03		2.21	300 m

Table 2. Continued

Shannon-Wiener Index				Ostracoda																												
H'	Variance H	Exp. H	HABITATS	*Cyprideis torosa*	*Candona angulata*	*Candona neglecta*	*Fabaeformiscandona* cf. *latens*	*Fabaformiscandona balatonica*	*Pseudocandona albicans*	*Ilyocypris bradyi*	*Ilyocypris brehmi*	*Ilyocypris inermis*	*Ilyocypris gibba*	*Cypria optalmica*	*Physocypria kraepellini*	*Cypridopsis vidua*	*Potamocypris similis*	*Potamocypris fulva*	*Potamocypris pallida*	*Heterocypris incongruens*	*Heterocypris salina*	*Heterocypris reptans*	*Psychrodromus olivaceus*	*Psychrodromus fontinalis*	*Herpetocypris chevreuxi*	*Stenocypris bolieki*	*Bradleytriebella tuberculata*	*Cypretta seurati*	*Limnocythere inopinata*	*Candonopsis* sp.	*Candona* sp.	Intervals
1.34	0.00	3.80	Creek							79												21										
			Pool																													
			Rice paddy								9					3					1					12						
			Spring							2	3									11												**8**
			Trough													164					8											
			%abund.							2.63	0.39					5.42				0.36	0.29	0.68				0.39						150 m
1.62	0.00	5.05	Creek							6			42								3										28	
			Pond								2																					
			Rice paddy													1					3					3	4					
			Stream							35													1						41			**13**
			Trough																													
			Water body							1	1																					
			Wetland	154																	2											
			%abund.	5						1.36	0.1		1.36			0.03					0.26		0.03			0.1	0.13		1.33		0.91	0 m
2.35	All Sample Index																															
0.22	Jackknife Std Error																															

among these eight groups by means of using Community Analysis Package 4 software. The software Species Diversity & Richness 4 (Seaby & Henderson, 2006) was used to calculate Shannon-Wiener Index values for each group. An appreciable collinearity between the environmental variables used in Canonical Correspondence Analysis (CCA) was tested by using Environmental Community Analysis II (ECOM II). When variables had a Variation Inflation Factor > 10 and R-squared close to 1 indicative of multicollinearity (ter Braak, 1995), we removed those of highly correlated variables from the analysis. Prior to CCA, Detrended Correspondence Analysis (DCA) was tested suitability of data for CCA. Accordingly, length of gradient (>3) in DCA indicates that the data can be considered acceptable for CCA. Our DCA gradient length was calculated as 3.69 suggesting the suitability of data for CCA. All data were tested with Monte Carlo Permutation tests (499 permutation) with a Forward Selection of Variables where rare species were removed before analyses to reduce multicollinearity and arc-effect (Birks, Line, Juggins, Stevenson, & ter Braak, 1990; ter Braak, 1995). CCA was used to determine the relationships between ostracod species and environmental variables. DCA and CCA were performed with software package CANOCO for windows 4.5. A non-parametric Spearman Rank Correlation analysis was performed to see possibilities of correlation between species and used environmental variables (IBM-SPSS Statistics Version 21). In all statistical analyses, adult individuals of the species with undamaged soft body parts and carapaces were used.

Results

A total of 3083 individuals belonging to 28 taxa (Table 2) were encountered from 76 of 97 sampling sites in the Sinop province. It is important to highlight the finding of nine bisexual populations of *Fabaeformiscandona balatonica* (1♂), *Ilyocypris bradyi* (3♂♂, 1022♀♀), *I. inermis* (2♂♂, 100♀♀), *Candona angulata* (2♂♂, 11♀♀), *C. neglecta* (5♂♂, 7♀♀), *Cyprideis torosa* (86♂♂, 68♀♀), *Psychrodromus fontinalis* (5♂♂, 109♀♀), *Cypria ophtalmica* (2♂♂, 62♀♀) and *Candonopsis* sp. (3♂♂). Species diversity fluctuated from sea level to 1152 m a.s.l. (Figure 2). Among the sites, maximum numbers of species reported from a spring (site 19; Figure 1) includes six species (*C. neglecta*, *I. bradyi*, *I. inermis*, *P. fontinalis*, *Potamocypris similis* and *P. fulva*) at 645 m a.s.l.

ANOSIM results exhibited no significant difference in species composition among eight groups with 150 m intervals (P>0.05). Of the species, *I. bradyi* was encountered in all elevational ranges when *Heterocypris incongruens*, *H. salina* and *Psychrodromus olivaceus* occurred in seven intervals (Table 2). However, nearly half (13) of the 28 taxa (e.g., *C. torosa*, *Bradleytriebella tuberculata*, *F. balatonica*, *Cypretta seurati*) were discovered once in one of the eight elevational ranges. Overall, species diversity increased towards the middle elevational ranges (e.g., 301-450 m with 14 taxa, 601-750 m with 13 taxa) when percentage abundance and numbers of sampled sites showed variability (Table 2). The highest Shannon-Wiener diversity index value (H'=1.87) was reported for the range of 601-750 m while the lowest (H'=1.02) was found between 1051 and 1200 m (Table 2).

The first two axes of CCA explained 53.40% of relationships between 11 species and 10 environmental variables (water temperature, organic, inorganic and total phosphates of sediment, pH, dissolved oxygen concentration, chloride, calcium, magnesium and sodium) with a variance of 13.40 (Table 3).

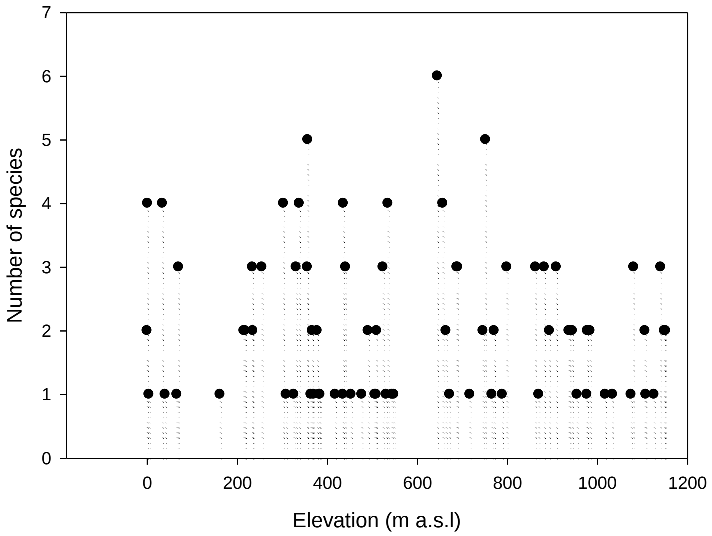

Figure 2. Distribution of numbers of species from sea level to 1152 m of elevation.

Three of the environmental variables (magnesium (Mg^{+2}, F=4.405, P=0.001), water temperature (Tw, F=3.099, P=0.011) and sediment organic phosphate (OrgPhos, F=3.278, P=0.027)) displayed strong effect on species distribution when the rest of them (pH (F=2.539, P=0.109), total phosphate (TotPhos, F=1.523, P=0.151), inorganic phosphates (InorgPhos, F=1.184, P=0.257), dissolved oxygen concentration (DO, F=1.305, P=0.263), chloride (Cl^{-1}, F=1.294, P=0.28), calcium (Ca^{+2}, F=0.964, P=0.432) and sodium (Na^{+1}, F=0.395, P=0.788)) did not show any significant effect. Eight species (*C. angulata*, *Cypridopsis vidua*, *H. salina*, *Ilyocypris brehmi*, *I. inermis*, *P. fulva*, *P. fontinalis* and *P. olivaceus*) located at the negative site of the second axis when three species (*H. incongruens*, *I. bradyi* and *Stenocypris bolieki*) were located on the positive site. While two species (*I. brehmi* and *H. salina*) showed close relationships with Mg^{+2} and Ca^{+2}, respectively, *I. bradyi* displayed a close relationship with InorgPhos. Two other species (*H. incongruens* and *S. bolieki*) are located on the site of Cl^{-1}, Na^{+1} and Tw but *C. angulata*, *P. fulva*, *I. inermis*, *P. fontinalis* and *P. olivaceus* are placed on the opposite site. Among the species, *C. vidua* did not show any close relationships with environmental variables in spite of settled on the site of Ca^{+2}, Mg^{+2}, pH and TotPhos (Figure 3).

The species with cosmopolitan characteristics showed significant correlations with some of the used variables (Table 4). Of which, *I. bradyi* exhibited a positive significant correlation with dissolved oxygen concentration (P<0.05) when negatively correlated with electrical conductivity (P<0.01), Na^{+2} and Mg^{+2} (P<0.01). *Heterocypris incongruens* and *P. olivaceus* were the only species with positive correlation to Ca^{+2}. Also, *P. fontinalis* (P<0.01), *H. incongruens* and *P. olivaceus* (P<0.05) displayed negative correlations with water temperature (Tw). Of the variables, elevation had a negative correlation with electrical conductivity (P<0.05), pH, water temperature, Na^{+2} and Cl^{-1} (P<0.01). Inorganic (P<0.05) and organic

Table 3. Summary table of Canonical Correspondence Analysis (CCA) for 11 species (with three or more occurrences) and 10 environmental variables. * represents the result of Detrended Correspondence Analysis (DCA).

Axes	1	2	3	4	Total inertia
*Lengths of gradient	5.29	3.69	2.32	2.89	
Eigenvalues	0.53	0.37	0.29	0.22	6.67
Species-environment correlations	0.77	0.68	0.59	0.62	
Cumulative percentage variance					
of species data	7.90	13.40	17.60	20.90	
of species-environment relation	31.50	53.40	70.40	83.40	
Sum of all eigenvalues					6.67
Sum of all canonical eigenvalues					1.67

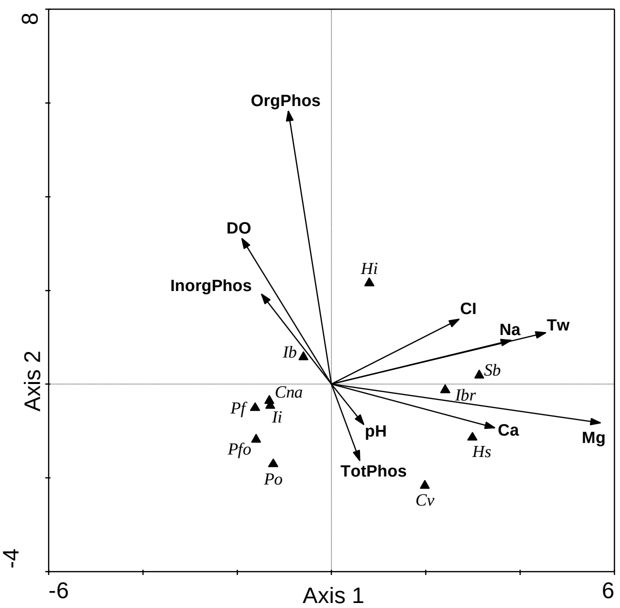

Figure 3. CCA diagram indicating distribution of 11 species (triangles) according to the effect of 10 environmental variables (arrows) from Sinop on the first and second axes. Abbreviations: *C. angulata* (Cna), *Cypridopsis vidua* (Cv), *H. salina* (Hs), *H. incongruens* (Hi), *Ilyocypris brehmi* (Ibr), *I. inermis* (Ii), *P. fulva* (Pf), *P. fontinalis* (Pfo), *P. olivaceus* (Po), *I. bradyi* (Ib) and *Stenocypris bolieki* (Sb).

Table 4. Spearman Rank Correlation Analysis results among 11 variables and five species. Abbreviations: electrical conductivity (EC), water temperature (Tw), dissolved oxygen concentration (DO), elevation (Elev), sodium (Na), magnesium (Mg), calcium (Ca), chloride (CI), organic phosphate (OrgPhosp), inorganic phosphate (InorgPhosp), *Heterocypris incongruens* (Hi), *Ilyocypris bradyi* (Ib), *I. inermis* (Ii), *Psychrodromus fontinalis* (Pfo) and *P. olivaceus* (Po). *Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Correlations												
			pH	EC	Tw	Na	Mg	Ca	CI	OrgPhosp	Ib	Hi
Spearman's rho	pH	Correlation Coefficient			.488**			-.416**		-.518**		-.526*
		Sig. (2-tailed)			.000			.000		.000		.021
		N			72			71		43		19
	DO	Correlation Coefficient			-.312**		-.254*				.356*	.467*
		Sig. (2-tailed)			.008		.032				.022	.044
		N			72		72				41	19
	EC	Correlation Coefficient			.349**	.576**	.695**	.367**	.590**		-.469**	
		Sig. (2-tailed)			.003	.000	.000	.002	.000		.002	
		N			72	71	72	71	71		41	-
	Tw	Correlation Coefficient	.488**	.349**		.308**	.252*		.345**			
		Sig. (2-tailed)	.000	.003		.009	.033		.003			
		N	72	72		71	72		71			
	Elev	Correlation Coefficient	-.318**	-.281*	-.375**	-.503**			-.598**			
		Sig. (2-tailed)	.007	.017	.001	.000			.000			
		N	72	72	72	71			71			
	Na	Correlation Coefficient		.576**	.308**		.555**		.706**		-.407**	
		Sig. (2-tailed)		.000	.009		.000		.000		.008	
		N		71	71		71		71		41	
	Mg	Correlation Coefficient		.695**	.252*	.555**			.454**		-.546**	
		Sig. (2-tailed)		.000	.033	.000			.000		.000	
		N		72	72	71			71		41	
	Ca	Correlation Coefficient	-.416**	.367**						.390**		.501*
		Sig. (2-tailed)	.000	.002						.010		.034
		N	71	71						43		18
	InorgPhosp	Correlation Coefficient						.328*				
		Sig. (2-tailed)						.032				
		N						43				
	Pfo	Correlation Coefficient	-.975**									
		Sig. (2-tailed)	.005									
		N	5									
	Po	Correlation Coefficient	-.589*		-.588*			.642**				
		Sig. (2-tailed)	.016		.017			.007				
		N	16		16			16				
	Ii	Correlation Coefficient		-.821*								
		Sig. (2-tailed)		,023								
		N		7								

(P<0.01) phosphate concentrations of the sediments and electrical conductivity (P<0.01) expressed a positive correlation with Ca^{+2} when pH (P<0.01) was negatively correlated (for more see Table 4).

Discussion

Before the present study, eight ostracod species (*C. neglecta*, *Ilyocypris biplicata*, *I. inermis*, *I. hartmanni C. torosa*, *Pseudocandona compressa*, *Cyclocypris ovum* and *Dolerocypris pellucida*) were known from Sinop (Ghetti, 1973; Kaleli, 1993; Kılıç, 2001). Of which, *I. biplicata* was accepted as a synonym of *I. gibba* in literature (Meisch, 2000). Therefore, the first four of these species were encountered in the present study and thus the remaining 24 taxa in the present study were firstly reported for the Sinop province (Table 2). Now, the numbers of ostracod taxa in Sinop are increased to 32 (30 living, 2 subfossils). Among the species, *Bradleytriebella tuberculata* is a new species (Figure 4) to Turkish Ostracoda fauna. Thus, the numbers of non-marine ostracod species in Turkey has increased to 144 species along with the recent checklist of Külköylüoğlu, Akdemir, Yavuzatmaca, and Yılmaz (2015).

Number of taxa per sample was lower in Sinop than the average numbers calculated from the previous studies in and out of Turkey (compare details in Table 5). Although increasing numbers of sampling sites tend to increase the numbers of species, we did not find such relationships. The number of taxa per sample can be elucidated by the quality of habitats, heterogeneity of geographical area and habitats types. For example, Rasouli, Aygen, and Külköylüoğlu (2014) had the highest taxa per sample since they collected 47 samples from 13 provinces in Turkey but Külköylüoğlu, Sarı, Akdemir,

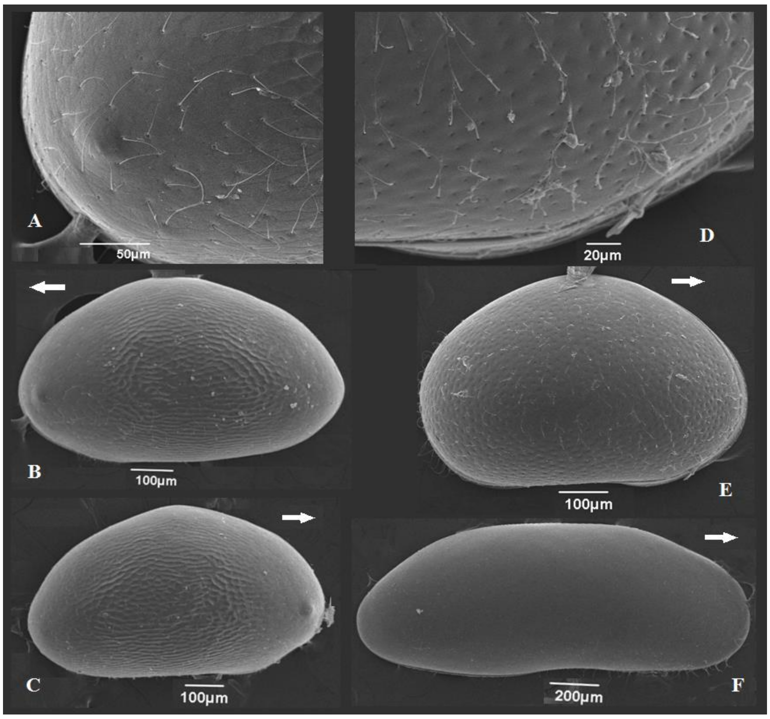

Figure 4. Scanning Electron Microscope images. A-C; *Bradleytriebella tuberculata*, A: anterior part of the left valve; B: outside view of left vale; C: outside view of right valve. D-E; *Cypretta seurati*, D: outside view of right valve; E: anterior part of right valve. F; *Stenocypris bolieki*, outside view of right valve. Arrows show the anterior side.

Table 5. Number of samples, taxa or species, and number of taxa or species per sample in some previous studies and their elevational ranges (as possible), and in the present study. * indicates studies in Turkey

Place	Number of samples	Number of taxa or species	Elevation range (m)	Number of species or taxa per sample	Citation
13 Provinces*	47	37	14-1723	0.79	Rasouli *et al.*, 2014
Kahramanmaraş*	95	46	432-1219	0.48	Külköylüoğlu *et al.*, 2012b
Gaziantep*	70	29	335-944	0.41	Akdemir *et al.*, 2016
Brazil	132	54	-	0.41	Higuti *et al.*, 2009
Germany	116	47	-	0.41	Viehberg, 2006
Belarus	156	62	≤220-230	0.40	Nagorskaya & Keyser, 2005
Van*	78	29	1659-2889	0.37	Külköylüoğlu *et al.*, 2012c
Northern Finland	38	14	32-673	0.37	Iglikowska & Namiotko, 2010
Erzincan*	89	32	840-1954	0.36	Akdemir & Külköylüoğlu, 2014
Adıyaman*	120	41	499-1383	0.34	Yavuzatmaca *et al.*, 2015
Burdur*	121	35	282-1538	0.29	Yavuzatmaca *et al.*, 2017
Sinop*	97	28	0-1152	0.29	This study
Çankırı*	130	37	549-1426	0.28	Külköylüoğlu *et al.*, 2016
Bolu*	141	40	-	0.28	Külköylüoğlu & Sarı, 2012
Diyarbakır*	90	23	530-1025	0.26	Akdemir & Külköylüoğlu, 2011
Eastern Iberian	117	28	5-1200	0.24	Mezquita *et al.*, 1999
NE Italy	320	74	1-1950	0.23	Pieri *et al.*, 2009
Ankara*	173	31	442-1518	0.18	Uçak *et al.*, 2014
Ordu*	166	26	0-1415	0.16	Külköylüoğlu *et al.*, 2012a
			Average	0.34	

Yavuzatmaca, and Altınbağ (2012a) had the lowest value where 166 samples were collected from one province. Some of those studies in Table 5 had similar results but effect of some other temporal (e.g., sampling season, time) and spatial factors (e.g., habitat conditions, disturbance, pollution, etc.) should also be considered critically.

The non-significant differences among the species composition of eight different elevational ranges are due to the common occurrences of "cosmoecious" species (e.g., *I. bradyi, H. incongruens*) almost in all ranges (Table 2). In addition, supportive approach of "Intermediate Disturbance Hypothesis" (Connell, 1978) was provided for the species diversity at different elevational ranges in the present study where the highest species diversity was reported progress to middle elevation when the diversity index values began to decrease at the low and high elevational ranges (Table 2). Our results coped with the results of some previous studies. For example, Külköylüoğlu, Yavuzatmaca, Akdemir, and Sarı (2012b) reported highest species number (20 spp.) at 801-1000 m when the range is 432-1219, and Uçak, Külköylüoğlu, Akdemir, and Başak (2014) noted the highest species diversity (16 spp.) at 841-940 and 941-1040 m where the range is 442-1518 m. In this case, the sampling habitats at low altitude are close to residential areas where they are under the human influence and so the conditions in these kinds of habitats fluctuate due to rate of anthropogenic activities. Although samplings were conducted at high elevational ranges far from residential areas, physico-chemical variables of water can be changed between the ranges. Since increase or decrease in elevation alters the temperature of surface water, any changes in temperature can be effective on physical, chemical and biological processes in water bodies (Chapman & Kimstach, 1996; Reeves, De Decker, & Halse, 2007). This is probably the case in the present study where water temperature showed positively significant correlations with pH, EC, Na^{+2}, Cl^{-1}, and Mg^{+2} but correlation was negative with elevation and DO (Table 4). Comparing to the conditions at low and high elevational ranges, conditions in mid elevations are relatively stable and can be considered better suitable sites for non-cosmopolitan species and also for cosmopolitans. Therefore, species diversity at mid elevations are found higher than at low and high elevations.

Canonical correspondence analysis reveals that local ostracod species composition in the Sinop province is significantly influenced by Mg^{+2}, water temperature and sediment's organic phosphate (Figure 3). Magnesium is one of the main trace element within the low magnesium calcium carbonated (in the form of calcite) carapaces of ostracod (Engstrom & Nelson, 1991; Anadón, Gliozzi, & Mazzini, 2002). Similar to our results, several previous studies pinpointed the effect of water temperature (Viehberg, 2006; Uçak *et al*., 2014) and Mg^{+2} (Viehberg, 2006) on species composition. Indeed, we found close relationship between Mg^{+2} and water temperature (Table 4). Implication of this result can be associated to the calcification process of ostracod carapaces during molting (Chivas, De Deckker, & Shelley, 1983; Carbonel & Töldener-Farmer, 1988). The effectiveness of sediment's organic phosphate can be related to the availability of food. This is because dissolved inorganic phosphate is absorbed by plants and subsequently incorporated in their body (Boyd, 2000). If one recognizes the role of ostracods in benthic food web and their feeding behaviour (e.g., dead and living plant parts, dead animal body) that may elucidate why organic phosphate concentration of sediments is important for the distribution of ostracods. Unfortunately, knowledge on the relationship between sediment and ostracods is scarce and further studies are necessary.

Ilyocypris bradyi is a well-known "cosmoecious" species (Külköylüoğlu, 2013). The common occurrences of the species in all elevational ranges in the present study (Table 2) corresponds with the wide tolerance ranges of species across different elevational ranges (0-3194 m) (Yavuzatmaca, Külköylüoğlu, & Yılmaz, 2017). Unlike Uçak *et al*. (2014), the species exhibited positive and negative correlations with DO and EC, respectively (Table 4, Figure 3). Also, it showed a close relationship with inorganic phosphate in CCA diagram (Figure 3) and had negative correlations with Na^{+1} and Mg^{+2} (Table 4). Li, Liu, Zhang, and Sun (2010) collected species from the Lake Qinghai water with Na^{+1} (0.003-2.662 g L^{-1}) and Mg^{+2} (0.015-0.743 g L^{-1}) contents and we collected species from waters with relatively high Na^{+1} (1.09-237.43 mg L^{-1}) and Mg^{+2} (0.75-60.23 mg L^{-1}) contents. *Ilyocypris inermis* and *I. brehmi* had lower percent abundances than *I. bradyi* (Table 2). The ordination of *I. inermis* on the opposing site of Na^{+1}, Cl^{-1} and water temperature in CCA diagram (Figure 3) support the previously noted negative correlation of species with electrical conductivity (Külköylüoğlu, Sarı & Akdemir, 2012c) and water temperature (Külköylüoğlu *et al*., 2013). The ecology of *I. brehmi* is not well known. Therefore, further studies are needed to make better comments about the ecological preferences of *I. brehmi*.

Another "cosmoecious" species, *P. olivaceus*, had the second highest abundance (Table 2). Mischke *et al*. (2014) revealed that *P. olivaceus* prefers habitats with low specific electrical conductivity (EC) and stable ion compositions. This finding was supported within the present study that *P. olivaceus* was positioned on the opposite site of Na^{+1} and Cl^{-1} contents in CCA diagram (Figure 3). In contrast, Külköylüoğlu *et al*. (2013) found a positively significant correlation of the species with EC. Unlike *P. olivaceus*, *P. fontinalis* had a low abundance value. As found in the present study, Külköylüoğlu and Sarı (2012) reported a negative significant correlation of species with pH. Although the negative relationships of *P. fontinalis* with water temperature (Uçak et al., 2014; the present study), wide range of water temperature (7.4-29.8 ºC) (Yavuzatmaca *et al*., 2017) where species found in literature showed that it can tolerate moderately high temperature.

The correlation of *H. incongruens* occurrences with high values of dissolved oxygen, conductivity and Ca^{+2} content (Van der Meeren, Almendinger, Ito, & Martens, 2010; Iglikowska and Namiotko, 2012) was reinforced by the findings of the present study in

Table 4. Lowest and highest Ca^{+2} content values as 49.64-210.14 mg L^{-1} for species were noted in the present study when Li *et al.* (2010) and Van der Meeren *et al.* (2010) reported 0.452-0.615 g L^{-1} and 0.1-62.5%, respectively. Also, Cusminsky, Pérez, Schwalb, and Whatley (2005) revealed 8.67-27.97 mg L^{-1} range of Ca^{+2} content for the species. Close relationship of *H. salina* with Ca^{+2} content herein (Figure 3) support the presence of species in the high Ca^{+2} content (average = 30.4%) in western Mongolia (Van der Meeren *et al.*, 2010). We encountered *H. salina* in waters with 6.07-210.14 mg L^{-1} Ca^{+2} content when Li *et al.* (2010) collected it in waters with 0.017-0.615 g L^{-1}. Unlike Valls, Rueda, and Mesquita-Joanes (2014) and Yavuzatmaca *et al.* (2017), we did not find any close relationships of *H. salina* with dissolved oxygen concentration (Figure 3). Mazzini *et al.* (2014) and Valls *et al.* (2014) revealed the presence of species in waters with 2.38-2.46 mg L^{-1} (but not specified) and 0.06±0.00-0.17±0.14 mg L^{-1} phosphate contents, respectively, when we gathered *H. salina* from water with 0.14-2.48 mg kg^{-1} inorganic and 0.025-0.23 mg kg^{-1} organic phosphates contents of sediment.

Like to our finding, Akdemir and Külköylüoğlu (2014) pinpointed the opposing relationship of *C. vidua* with dissolved oxygen content but Hussein, Obuid-Allah, Mahmoud, and Fangary (2004) reported a positive correlation. Range of dissolved oxygen for the species in literature was expand with the present study (0.6-19 mg L^{-1} (the present study; Külköylüoğlu, 2013)). Additionally, Pieri, Vandekerkhove, and Goi (2012) and Mazzini *et al.* (2014) encountered species in water with 0.08-0.24 mg L^{-1} total phosphorus in Ledra River (not specified) and 0.48-2.55 mg L^{-1} phosphate in Caffarella Valley (Italy), respectively, while we collected it in waters with total phosphate (0.104-0.64 mg kg^{-1}), inorganic phosphate (0.251-2.48 mg kg^{-1}) and organic phosphate (0.022-0.23 mg kg^{-1}) of sediments. Moreover, the range of Mg^{+2} (6.04-56 mg L^{-1} (the present study; Palacios-Fest and Dettman, 2001)) and Ca^{+2} (34.10-258 mg L^{-} (the present study; Palacios-Fest and Dettman, 2001)) for *C. vidua* was also enlarged.

Meisch (2000) stated that *Candona angulata* prefers slightly salty waters (0.2-14 ‰) and this statement was supported by the ordination of species on site of Cl^{-1}, Na^{+1} and water temperature in the present study. We have poor information about the ecology of *P. fulva*. Although species located on the opposing site of water temperature in the present study (Figure 3), *P. fulva* has a wide range of water temperature in literature (1.56-27.40°C (Külköylüoğlu *et al.*, 2007; Sarı, 2007)).

There is almost no any ecological and biological information about *Stenocypris bolieki*. Minimum and maximum values of some variables of aquatic bodies where *S. bolieki* occurred in the present study were as followings water temperature (20.08-30°C), dissolved oxygen (0.6-2.03 mg L^{-1}), pH (7.71-8.25), electrical conductivity (568-1050 µS cm^{-1}), elevation (34-303 m), Na^{+1} (12.04-45.40 mg L^{-1}), Mg^{+2} (6.07-28.72 mg L^{-1}), Ca^{+2} (65.67-89.35 mg L^{-1}) and Cl^{-1} (4.31-24.97 mg L^{-1}).

In conclusion, with the findings of the present study, numbers of ostracod taxa have been increased to 32 taxa in Sinop. Number of taxa/species per sample and species diversity are inclined by habitat quality and geographical heterogeneity rather than the number of sampling sites. Also, anthropogenic impact and physico-chemical changes at low and high elevations, respectively, affect the species composition. This causes the common presence of cosmopolitans in almost all elevational ranges when non-cosmopolitans seem to be distributed in undisturbed low and middle elevational ranges. In contrast to the statement of Smith (1993) as sensitivity of ostracod's distribution to anions not cations, we found the significant effect of Mg^{+2} along with water temperature and total phosphate content of sediments. Accordingly, the number of studies on ostracods coupling with water and sediment analyses should be increased to provide better understanding of their characteristic in future work.

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