Ann. Limnol. - Int. J. Lim. 2018, 54, 26
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https://doi.org/10.1051/limn/2018017

Available online at:
www.limnology-journal.org

Research Article

# Ecological classification of the freshwater Ostracoda (Crustacea) based on physicochemical properties of waters and habitat preferences

Okan Külköylüoğlu[1,*], Mehmet Yavuzatmaca[1], Derya Akdemir[2], Ebru Çelen[1] and Nurhayat Dalkıran[3]

[1] Abant İzzet Baysal University, Faculty of Arts and Science, Department of Biology, Bolu, Turkey
[2] Merdivenköy Mahallesi, Sair Arsi Caddesi, No:16/14, Kadıköy, İstanbul, Turkey
[3] Uludağ University, Faculty of Arts and Science, Department of Biology, Bursa, Turkey

Received: 27 March 2018; Accepted: 24 May 2018

**Abstract –** The relationship between ecological characteristic of freshwater ostracods and their habitat preferences has been a critical issue for understanding of both current and past aquatic conditions. To evaluate this idea, 121 water bodies with 11 different habitat types were randomly sampled in the province of Kütahya. Water quality measurements indicated high to low ($Ca^{2+} > Mg^{2+} > Na^{+}$) cations and relatively low ($SO_4{}^{2-} > Cl^{-} > F^{-}$) anion concentrations with $Ca^{2+}$ being the dominant ion. Sixteen of 23 species were new reports for the area. Alpha diversity ($H' = 3.64$) was found relatively high. Four most abundant species with ca. 93% of similarities contributed highest alpha values in warm to cooler (lower than 25 °C), alkaline (pH 8.22), and fresh to slightly brackish waters. *Heterocypris salina* and *Ilyocypris bradyi* also revealed the highest tolerances for electrical conductivity. Based on habitat type, species were clustered into three main groups (I–III). Canonical Correspondence Analyses explained about 57.4% of correlation between species and environmental variables. Redox potential, pH, water temperature and electrical conductivity were found to be the most effective factors on species occurrences while habitat type and dissolved oxygen were not effective. Total number of species showed strong negative and positive relationships with water temperature and dissolved oxygen, respectively. Results clearly showed that cosmopolitan species exhibited relatively wide tolerance ranges to different environmental variables. Accordingly, having wide tolerance ranges seems to provide advantages to cosmopolitan species, increasing their survival chances in a variety of habitats.

**Keywords:** Ecological tolerance and optimum / habitat preference / diversity / abundance / major ions

## 1 Introduction

Species of Ostracoda, which are small microscopic (0.3–7 mm) invertebrates, are one of the most widely dispersed groups of Crustacea. Living forms are distributed from ca. 5000 m deep in ocean (Benson, 1972; Brandão and Yasuhara, 2013) and up to over 4000 m (Laprida *et al.*, 2006; Mischke *et al.*, 2007) and 6000 m in mountain lakes (Pinto, 2013). They can be found in a variety of aquatic habitats from thermal waters (Külköylüoğlu *et al.*, 2003) to cold springs, rivers, creeks, ponds, lakes, troughs and canals. While several species are known to have high levels of tolerances to different environmental variables, some species are considered to have habitat specific adaptive values and can be used as indicator species for particular habitats and conditions. For example, while *Heterocypris incongruens* (Ramdohr, 1808) is a cosmoecious species that is widely distributed with wide tolerances to some of those major environmental variables (*e.g.*, pH, water temperature, dissolved oxygen, etc.) (Külköylüoğlu, 2013), *Thermopsis thermophila* Külköylüoğlu, Meisch, Rust 2003, a thermophilic ostracod with a narrow temperature range of 36–55 °C, has only been found from thermal springs in the Great Basin area (USA) (Külköylüoğlu *et al.*, 2003). Despite their importance, freshwater ostracods are not studied extensively and we have a limited knowledge about their water and/or habitat preferences and requirements. In contrast, habitat preference and requirements of species (*i.e.*, ostracods) constitute an important topic for several reasons; they can (1) provide understanding of habitat (water) qualities and/or conditions influenced by natural or anthropogenic factors, (2) be used to estimate past, present and future habitat conditions and species

*Corresponding author: okankul@gmail.com

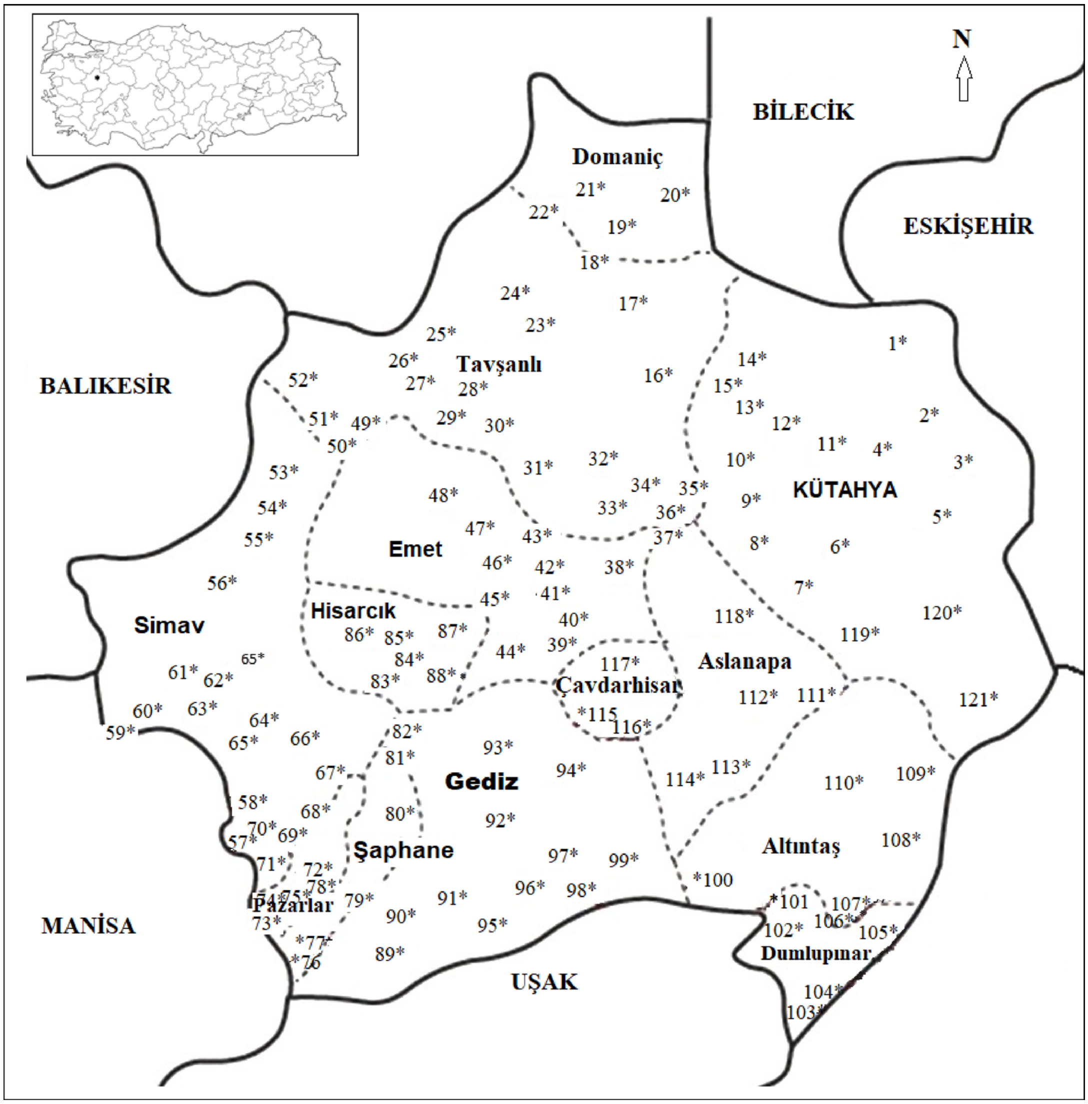


**Fig. 1.** Sampling sites of 121 different sites in Kütahya, Turkey.

diversities, (3) help monitoring studies and/or conservation issues by means of calculating species co-occurrence patterns, (4) obtain possibilities for accounting abundance and/or diversity measurements, (5) open ways to recognize species role in the community and ecosystems, (6) increase the possibility of calculating their ecological tolerance and optimum values for different environmental factors, and (7) explain species geographical distributional patterns among the habitats (this study). Additionally, it is useful to understand whether certain species of ostracods may prefer (or require) particular type of habitat(s). The importance of this view implies that when species' habitat preferences are known (if there is any), this information can also be used in palaeontological and palaeogeographical studies for the purpose of reconstruction of the past history of habitats.

There are a few previous studies (Gülen, 1977; 1985a, b; Gülen and Altınsaçlı, 1999) in the area of Turkey but these studies are based on one-time sampling efforts from a few sites, and there is no extensive studies related to habitat preferences and ecology of freshwater ostracods. Accordingly, the aims of the present study were (i) to understand ostracod species' habitat preferences, (ii) to describe their ecological (physical and chemical preferences) characterization, (iii) to examine the most effective factors on species occurrences amid habitat types, and (vi) to estimate species ecological tolerance and optimum levels in different habitats along with providing species diversity measurements.

## 2 Materials and methods

### 2.1 Sampling and methodology

Our sampling sites were randomly visited within the borders of the province Kütahya (ca. 11889 $km^2$ of surface area, covering about 1.5% of Turkey), located in the Aegean region of Turkey (Fig. 1). All samples were gained from shallow (<100 cm of depth) and littoral zones of the aquatic habitats. Samples were collected from 121 sites with 11 different aquatic habitat types (lake, reservoir, pond, wetland, ditch, trough, river, stream, creek, canal and thermal spring)

**Table 1.** Mean, maximum, minimum and standard deviations (SdDev) of the environmental variables reported from sampling sites are shown. Higher values of deviations more than the mean values for those variables are italicized. *Abbreviations*: TPh (total phosphate), InPh (inorganic phosphate), OrPh (organic phosphate). Other abbreviations are provided in materials and methods section.

| | pH | DO | EC | Tw | SHE | Elev | $Na^+$ | $K^+$ | $Mg^{2+}$ | $Li^+$ | $Ca^{2+}$ | $F^-$ | $Cl^-$ | $NO_2^-$ | $SO_2^{2-}$ | TPh | InPh | OrPh |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mean | 8.22 | 10.5 | 657 | 17.4 | 217 | 951 | 15.1 | 5.38 | 30.7 | 0.05 | 69.6 | 0.18 | 12.1 | 8.26 | 149 | 0.46 | 0.39 | 0.09 |
| Minimum | 6.55 | 1.46 | 80.2 | 10.8 | 50.2 | 460 | 0 | 0 | 0.68 | 0 | 8.89 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Maximum | 9.63 | 18.6 | 3097 | 36.9 | 292 | 1380 | 140 | 89.1 | 110 | 1.65 | 522 | 2.73 | 104 | 112 | 2480 | 2.71 | 2.52 | 0.38 |
| SdDev | 0.5 | 3.81 | 452 | 3.45 | 37.8 | 184 | *21.2* | *11.8* | 22.5 | *0.19* | 56.2 | *0.39* | *14.6* | *18.3* | *354* | 0.46 | *0.43* | *0.1* |

using a hand net (200 µm mesh size) between 19–22 September 2014, and were immediately fixed within 70% ethanol in 250 ml plastic containers. In the laboratory, the samples were filtered using standard sieves (0.5, 1.0, 1.5 and 2.0 mm mesh size) under tap water and transferred into 70% ethanol for future research. Ostracod specimens were separated from other materials with fine needles under a Meiji-Techno stereomicroscope. Each specimen' soft body parts and carapaces were separated in lactophenol solution after body length was measured for taxonomic description. Each slide was labeled and kept in the laboratory. Olympus-CX4 1 light microscope was used during species identification which was basically done by following the taxonomic keys of Meisch (2000) and Karanovic (2012). Each sample labeled with a code was deposited at the Limnology Laboratory of Abant İzzet Baysal University, Bolu, Turkey and can be available upon request.

Before sampling, GARMIN etrex Vista H GPS system was used to measure geographical information for elevation (elev.) and coordinates about each of sampling sites. Then after, all water samples were collected to prevent possible disturbance for water measurements. Seven major water variables (pH, oxidation-reduction potential (SHE, mV), water temperature (Tw, °C), dissolved oxygen concentration (DO, mg/L), percent oxygen saturation (%sat.), electrical conductivity (EC, µS/cm), salinity (ppt)) and atmospheric pressure (mmHg) were measured with YSI-Professional Plus. Kestrel-3000 model anemometer was used to record air temperature (Ta, °C), relative humidity (%), heat index (HI, °C), wind chill (w. chill, °C), wind speed (m/s), and dew point (Dp, °C) *in situ* (Tab. 1). For the major water ion concentrations, 100 ml water samples were collected from each sampling site in clean plastic bottles, preserved in cool containers, and analyzed in the laboratory for cations (sodium ($Na^+$), ammonium ($NH_4^+$), potassium ($K^+$), magnesium ($Mg^{2+}$), lithium ($Li^+$), calcium ($Ca^{2+}$)) and anions (chloride ($Cl^-$), nitrite ($NO^{2-}$), nitrate ($NO_3^{2-}$), fluoride ($F^-$), phosphate ($PO_4^{3-}$), bromide ($Br^-$), and sulphate ($SO_4^{2-}$)). Note that some of those variables are not listed in Table 1 but will be available upon request. Detailed information about the procedure of major ion analyses can be found in Yavuzatmaca *et al.* (2017a). From each site, we also collected sediment samples in plastic Eppendorf vials for organic and inorganic phosphate and total phosphate (mg kg$^-$) analyses as described in Ruban *et al.* (1999). Meisch (2000) performed ecological characterization of ostracod species following Hiller (1972) and Hartmann and Hiller (1977). For the species salinity tolerance/preference, he adapted the Venice-system which is also adapted in the current study. According to this system, species were classified with temperature, calcium contents, and salinity and chloride contents (see details in Meisch, 2000). However, the Venice system does not show the water temperature ranges for freshwater habitats. Therefore, we used the temperature ranges ((very cold < 12.8 °C), (>12.8 °C cold ≤ 18 °C), (>18 cold-cool ≤ 21 °C), or (warm ≤ 21 °C)) (Chu *et al.*, 2009; Olivero-Sheldon *et al.*, 2014).

## 2.2 Statistical analyses

Alpha diversity of Shannon-Wiener index values were calculated with the Species Diversity and Richness 4 (SDR) program (Seaby and Henderson, 2006). Species percentage contribution and dis/similarities in habitats were analyzed with the analysis of similarities (ANOSIM) and analysis of similarity percentage (SIMPER) along with the Bray-Curtis test. In each of the branching point, habitat(s) effective on species occurrence are illustrated with their effective value. Both analyses were run in Community Analysis Package (CAP 4.1.3.) statistical program (Seaby and Henderson, 2007).

Estimatination of the most effective environmental variables and their correlation with ostracod species was made with the program CANOCO for windows 4.5 (ter Braak and Šmilauer, 2002) where quantitative data were used for the species. Results were tested with Monte Carlo Permutation tests (499 run) while multicollinearity test was applied to download rare species to eliminate possible consequences of multi-collinearity (Birks *et al.*, 1990; ter Braak, 1995). Accordingly, variables with higher inflation factor (VIF > 10) representing a possibility of multicollinearity were removed from the analyses. As suggested (ter Braak and Barendregt, 1986; ter Braak, 1995), before CCA, we tested suitability of our data for CCA with detrended correspondence analysis whose length of gradient was 6.58 indicating the data was suitable for CCA. Species clustering relationships based on habitat type were analyzed with unweighted pair group mean averages (UPGMA) where Jaccard's coefficient was preferred to use after data were log(e) transformed in Multi Variate Statistical Package version 3.1. (Kovach, 1998). Pearson correlation analysis (SPSS program version 17) (SPSS, 2008) was applied to evaluate possible associations among 22 environmental variables and major ions along with numbers of species. Species ecological tolerance and optimum estimates were calculated with C2 program (Juggins, 2003), where we used species found from three or more different sites. Ternary and scattered plots used to estimate distributional patterns of species with some of those major ions were obtained from JMP ® Pro13.0 program. We only used adult individuals

**Table 2.** Distribution of numbers of species (NuSpp) are shown from 11 different habitat types. Numbers of habitat type (NuHabtype) indicate numbers of samplings from that of particular habitats. For example, three samplings were done from three different lakes with two species. Two well samples are not shown in this table due to lack of ostracods from these two sites. New reports are shown in bold. Abbreviations: CC, (***Candona candida***); CN, (*C. neglecta*); COP, (*Cypria ophtalmica*); CV, (***Cypridopsis vidua***); DS, (***Darwinula stevensoni***); HI, (***Heterocypris incongruens***); HAS, (*H. salina*); HR, (***Herpetocypris reptans***); HC, (*H. chevreuxi*); IBR, (***Ilyocypris bradyi***); IG, (*I. gibba)*; IH, (***I. hartmanni***); LI, (***Limnocythere inopinata*); PK, (*Physocypria kraepelini*);** PFU, (***Potamocypris fulva***); PP, (***P. pallida***); PU, (***P. unicaudata***); PV, (***P. variegata***); PVI, (***P. villosa***); PZE, (*Prionocypris zenkeri*); PFO, (***Psychrodromus fontinalis***); PO, (*P. olivaceus*); TC, (***Trajancypris clavata***).

| Habitat type | NuHabtype | Species | NuSpp |
|---|---|---|---|
| Lake | 3 | IBR, LI | 2 |
| Creek | 7 | CC, CN, DS, HSA, IBR, IH, PV, PZE, PO | 9 |
| Trough | 60 | CV, DS, HR, HC, HI, HSA, IBR, IH, PFU, PP, PV, PVI, PZE, PFO, PO | 15 |
| Reservoir | 16 | COP, DS, HI, IBR, IG, LI, PK, PU, PV, PZE | 10 |
| Stream | 7 | COP,HSA, IBR, IH, PFU, PZE, PO | 7 |
| Pond | 10 | CV, HR, HI, HSA, IBR, LI, PV, PVI, PO | 9 |
| River | 2 | HSA, IH | 2 |
| Canal | 1 | CV, HI, IBR | 3 |
| Water body | 9 | DS, HI, HSA, IBR, IG, PO, TC | 7 |
| Wetland | 1 | HI, IBR | 2 |
| Thermal spring | 3 | HSA | 1 |

during the statistical analyses. Juveniles, carapaces and/or damaged individuals were not used.

## 3 Results

In general, species were found in cool (>18 °C) to warmer (≥21 °C), alkaline (pH 8.22), and fresh to slightly brackish waters. Water quality measurements (Tab. 1) indicated low to high (high $Ca^{2+}$ > low $Mg^{2+}$ > very low $Na^+$) cations and relatively low (low $SO_4^{2-}$ > very low $Cl^-$ > not significant $F^-$) anion concentrations where $Ca^{2+}$ was the dominant ion.

Total of 23 species (***Candona candida*** (O.F. Müller, 1776); *C. neglecta* Sars, 1887; *Cypria ophtalmica* (Jurine, 1820); ***Cypridopsis vidua*** (O.F. Müller, 1776); ***Darwinula stevensoni*** (Brady and Robertson, 1870); ***H. incongruens*** (Ramdohr, 1808); *Heterocypris salina* (Brady, 1868); ***Herpetocypris reptans*** (Baird, 1835); *H. chevreuxi* (Sars, 1896); ***Ilyocypris bradyi*** Sars, 1890; *I. gibba* (Ramdohr, 1808); ***I. hartmanni*** Lerner-Seggev, 1968; ***Limnocythere inopinata*** (Baird, 1843); ***Physocypria kraepelini*** G. W. Müller, 1903; ***Potamocypris fulva*** (Brady, 1868); ***P. pallida*** Alm, 1914; ***P. unicaudata*** Schäfer, 1943; ***Potamocypris variegata*** (Brady and Norman, 1889); ***Potamocypris villosa*** (Jurine, 1820); *Prionocypris zenkeri* (Chyzer and Toth, 1858); ***Psychrodromus fontinalis*** (Wolf, 1920); *Psychrodromus olivaceus* (Brady and Norman, 1889); ***Trajancypris clavata*** (Baird, 1838)) belonging to 13 genera collected from 11 different aquatic habitat types were found from 91 of 121 sampling sites in Kütahya between 19 and 22 September 2014 (Tab. 2). Sixteen species (shown in bold above) were new reports for the province.

Shannon-Wiener alpha diversity index value ($H'$) was 3.64 with relatively low error term (Jackknife Std Error 0.283). Four species (*H. salina*, *H. incongruens, P. olivaceus*, *I. bradyi*) creating more than 93% of similarities contributed higher alpha values (2.625, 2.434, 2.301, 2.159, respectively) than other species. ANOSIM results revealed that unassigned group averages of 78.65% of the contribution of similarities were provided by 16 most frequently occurring species. Accordingly, finding a negative $R$ value, (sample statistics = −1, $p$ = 0.001, $n$ = 89 observations) suggested that there were no significant differences between the species of the groups, implying that most similar samples were independently found outside of those groups. All the four species exhibited equal or higher tolerance values for pH, dissolved oxygen and water temperature than the average values of the total of 12 most abundant species (Tab. 3). Of which, *H. salina*, *I. bradyi* and *L. inopinata* also revealed the highest tolerances for electrical conductivity while some other species (*e.g.*, *P. variegata* for dissolved oxygen, *P. villosa* and *D. stevensoni* for water temperature) also showed high tolerance levels to different variables. These results may shed light into the fact that these species play a dominant role in a variety of habitats due to their high levels of ecological tolerances.

Among these species, *H. incongruens* was mostly found in waters with high $Ca^{2+}$ concentrations between 60 and 80% but low $Na^+ + K^+$ and $Mg^{2+}$ concentrations (<40%) while *H. salina* was collected from waters with higher $Ca^{2+}$ between 65 and 90% of dominancy but medium $Na^+ + K^+$ (40–80%) and relatively high (>60%) of $Mg^{2+}$ (Fig. 2). Individuals of *I. bradyi* were encountered from the waters with high $Ca^{2+}$ concentrations between 60 and 85% of dominancy but low $Na^+ + K^+$ (<30%) and $Mg^{2+}$ (<40%). Another species, *P. olivaceus* was also common in waters with a wide range of low to high $Ca^{2+}$ concentrations from 30 to 90% of dominancy but very low $Na^+ + K^+$ (<20%) and $Mg^{2+}$ (≥60%) (Fig. 3). These species clearly showed higher tolerance levels for dissolved oxygen and water temperature than the mean of total of all species although a few species displayed different levels of tolerance and optimum estimates (see discussion below) (Tab. 3).

UPGMA diagram illustrated three main clustering groups (Fig. 4). In which, regardless of their taxonomic levels, species with common features tend to be clustered in the same group. Groups I and III mostly consisted of species

**Table 3.** Optimum (Opt) and tolerance (Tol) values of 12 most abundant species occurred more than three times from different habitats. Abbreviations: Numb, numbers of occurrences; Max, maximum numbers of individuals; N2, Hill's coefficient. Other abbreviations are given in Materials and Methods.

| Species | Numb | Max | N2 | pH | | DO | | EC | | Tw | | Elev | | SHE | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Opt | Tol | Opt | Tol | Opt | Tol | Opt | Tol | Opt | Tol | Opt | Tol |
| *Darwinula stevensoni* | 4 | 214 | 1.15 | 8.29 | 0.32 | 7.95 | 1.38 | 261 | 239 | 18 | 2.63 | 859 | 74.9 | 230 | 79.4 |
| *Heterocypris incongruens* | 30 | 324 | 5.62 | 7.86 | 0.70 | 9.54 | 3.22 | 484 | 171 | 16.7 | 2.51 | 769 | 144 | 222 | 29.9 |
| *Heterocypris salina* | 25 | 440 | 8.79 | 7.99 | 0.45 | 12.2 | 3.66 | 767 | 356 | 17.6 | 2.66 | 927 | 180 | 222 | 25 |
| *Ilyocypris bradyi* | 29 | 224 | 5.17 | 7.98 | 0.41 | 11.1 | 4.1 | 549 | 248 | 18.8 | 2.55 | 1064 | 112 | 226 | 14.2 |
| *Ilyocypris hartmanni* | 4 | 121 | 1.84 | 8.3 | 0.32 | 9.67 | 1.21 | 994 | 234 | 20.7 | 0.33 | 662 | 329 | 135 | 48.5 |
| *Limnocythere inopinata* | 5 | 16 | 2.32 | 8.83 | 0.31 | 10.7 | 2.42 | 603 | 319 | 16.8 | 3.6 | 905 | 97.9 | 223 | 10.1 |
| *Potamocypris fulva* | 4 | 92 | 2.15 | 8.56 | 0.2 | 13.4 | 1.13 | 379 | 141 | 13.5 | 1.91 | 1053 | 93.3 | 231 | 21 |
| *Potamocypris variegata* | 7 | 450 | 2.5 | 7.71 | 0.69 | 11.4 | 4.62 | 515 | 236 | 17.2 | 1.26 | 746 | 107 | 216 | 38.3 |
| *Potamocypris villosa* | 11 | 295 | 3.41 | 8.73 | 0.75 | 14.9 | 3.05 | 387 | 218 | 17.8 | 2.93 | 868 | 326 | 207 | 15.1 |
| *Prionocypris zenkeri* | 11 | 204 | 2.3 | 7.8 | 0.23 | 8.36 | 2.28 | 559 | 157 | 13.8 | 1.54 | 1063 | 88.2 | 176 | 76.2 |
| *Psychrodromus fontinalis* | 4 | 25 | 2.81 | 8.31 | 0.19 | 12.2 | 1.63 | 374 | 148 | 18.1 | 1.83 | 1178 | 60.8 | 233 | 5.29 |
| *Psychrodromus olivaceus* | 25 | 244 | 5.6 | 8.12 | 0.41 | 10.8 | 2.88 | 474 | 180 | 15 | 2.29 | 1158 | 243 | 239 | 21.1 |
| **Mean** | 13.25 | 220.8 | 3.63 | 8.21 | 0.41 | 11.02 | 2.63 | 528.8 | 220.6 | 17 | 2.17 | 937.7 | 154.7 | 213.3 | 32.01 |

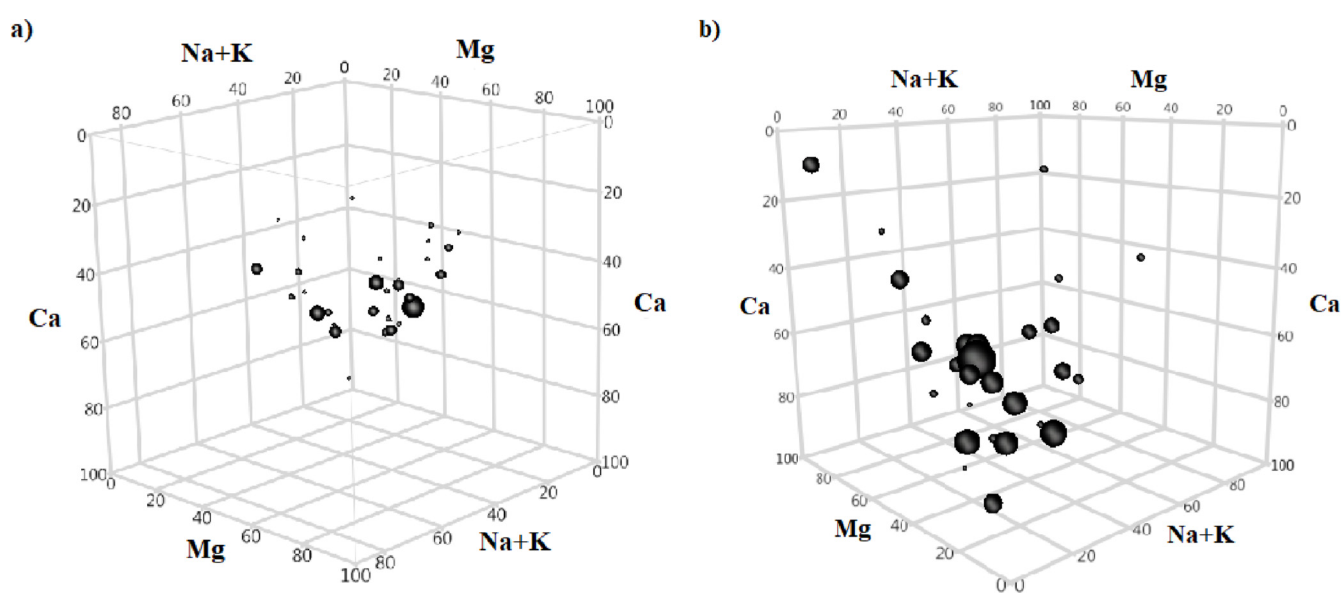


**Fig. 2.** Occurrence of *H. incongruens* (a) and *H. salina* (b) in waters with major cations ($Mg^{2+}$, $Ca^{2+}$ and $Na^{+} + K^{+}$). Values are in percentage.

with rare and/or low occurrences while most frequently occurring common species were generally clustered in group II. Except a few rare species (*e.g.*, *L. inopinata*, *C. neglecta*, *C. candida*), most ostracods showed no specific habitat preferences where habitat type was not effective on species occurrences ($p > 0.05$).

According to CCA results (Tab. 4, Fig. 5), four variables (Tw, EC SHE, pH) were found to be the most effective factors on species occurrences ($p < 0.05$) while 57.4% of correlation between species and environmental variables were explained with relatively low cumulative percent variation (6.4%) by the first two axes. Pearson correlation analyses showed strong positive and negative correlation between numbers of species and dissolved oxygen ($p = 0.002$) and water temperature ($p = 0.015$), respectively. Most of these common species showed relatively wide tolerance ranges to five major environmental variables (Tab. 3).

## 4 Discussion and conclusion

Until this study, 15 ostracod species were known from Kütahya. These species were mostly reported from hot springs, ponds, creeks, rivers, and swamps (Gülen, 1977; 1985a, b; Gülen and Altınsaçlı, 1999) of particular areas; therefore, they did not represent whole faunal richness of the area. In contrast, during the present study, sampling from all 13 towns, we reported 23 living ostracods from 11 different habitats, of which, 16 were new reports (Tab. 2). During the present study, we did not find eight species (*Cypris pubera*, O.F. Müller,

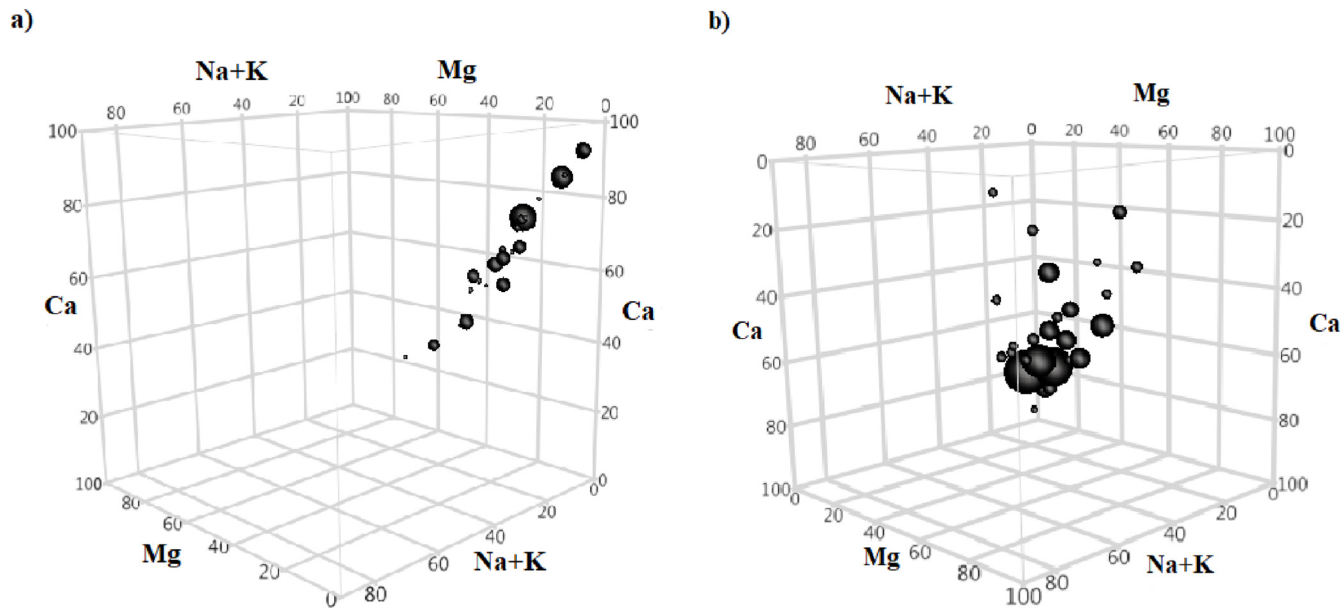


**Fig. 3.** Occurrence of (a) *P. olivaceus* and *I. bradyi* (b) in waters with major cations ($Mg^{2+}$, $Ca^{2+}$ and $Na^{+}+K^{+}$). Values are in percentage.

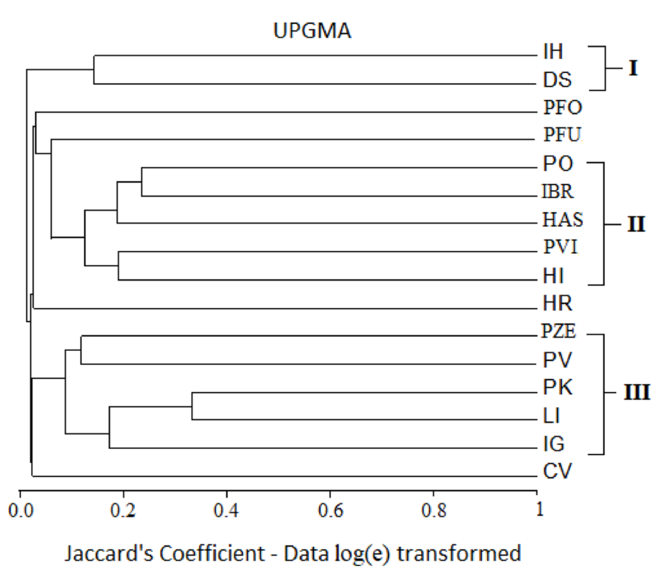


**Fig. 4.** UPGMA dendrogram shows clustering relationships among 16 species within three main groups (I–III).

1776; *Cyclocypris ovum* (Jurine, 1820); *Cypretta dubiosa* (Daday, 1901); *Dolerocypris sinensis* (Sars, 1903); *Eucypris virens* (Jurine, 1820); *Ilyocypris decipiens* Masi, 1905; *Ilyocypris divisa* Kliei, 1926; *Tonnacypris lutaria* (Koch, 1838)) which are previously reported from the area. In summary, the area consists of 31 ostracod species at the moment. Comparing to other studies, this number being relatively higher than many other provinces in Turkey suggests potentially high species diversity of the area. Indeed, finding high alpha diversity index value (3.64) supports this view. However, four most frequently occurring species (*H. salina*, *H. incongruens*, *P. olivaceus*, *I. bradyi*) seemed to be responsible for the majority of the diversity values (2.625, 2.434, 2.301, 2.159, respectively). It is probable that these so called "cosmoecious species" (Külköylüoğlu, 2013) are found in a variety of habitats with wide ranges of geographical distributions (Mezquita *et al.*, 1999a, b; Meisch, 2000; Külköylüoğlu, 2003; Van der Meeren et al., 2010; Yavuzatmaca *et al.*, 2017a, b). Their wide distribution may probably be related to high tolerance levels (Külköylüoğlu, 2003; Iglikowska and Namiotko, 2012). This was actually the case in the present study that our results confirm those of earlier and contemporary studies in different taxonomic groups. For example, Heino and Grönroos (2017) recently showed that while very rare stream insect species did not have much contribution to the local beta diversity, species occurring most frequently in many sites with high abundance and variation were able to provide the most contribution to the local beta diversity. Also, finding of no significant effect of habitat types and reporting species from a variety of habitats may suggest that these species may not have specific habitat preferences in our study.

UPGMA dendrogram (Fig. 4) clustered species into three main groups based on habitat type where most common species tend to be clustered in the second (II) group. It should be underlined that all groups consist of different species of different genera, suggesting no specific habitat preferences at the generic level. Two other groups (I, III) included more of those species with lower and/or rare occurrences. There are two species in the first group (I) while group III is divided into two subgroups. Of the two species in group I, *D. stevensoni* has a wide geographical distribution found from lentic to lotic habitats. In contrast, *I. hartmanni* is one of the rarest species and there is not much information about its ecological characteristics.

We found four variables (Tw, EC, SHE, pH) effective on species occurrence while Tw was negatively but strongly correlated to the numbers of species (Tab. 4, Fig. 5). Implication of this finding suggests increasing water temperature may be responsible for gradual reduction in the numbers of species. This

**Table 4.** Summary table of CCA results.

| Axes | 1 | 2 | 3 | 4 | Total inertia |
|---|---|---|---|---|---|
| Lengths of gradient | 6.586 | 3.955 | 5.215 | 4.967 | 6.394 |
| Eigenvalues | 0.243 | 0.164 | 0.133 | 0.097 | |
| Species-environment correlations | 0.641 | 0.544 | 0.560 | 0.425 | |
| Cumulative percentage variance | | | | | |
| of species data | 3.8 | 6.4 | 8.4 | 10.0 | |
| of species-environment relation | 34.2 | 57.4 | 76.1 | 89.9 | |
| Sum of all eigenvalues | | | | | 6.394 |
| Sum of all canonical eigenvalues | | | | | 0.709 |

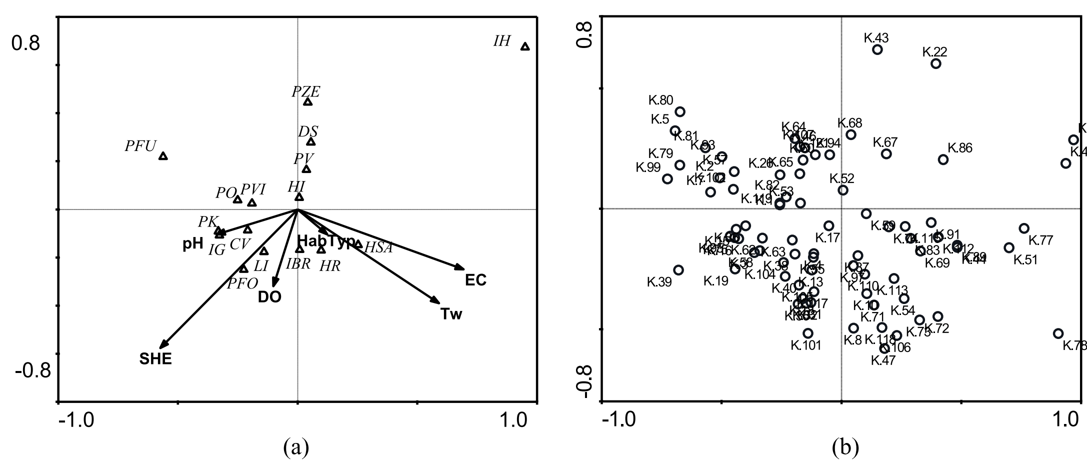


**Fig. 5.** (a) CaCA diagram shows distribution of 16 species and 6 environmental vairables measured in Kütahya. Species abbreviations are given in Table 2, (b) CbCA diagram shows distribution of sampling sites with species occurred three or more times in Kütahya.

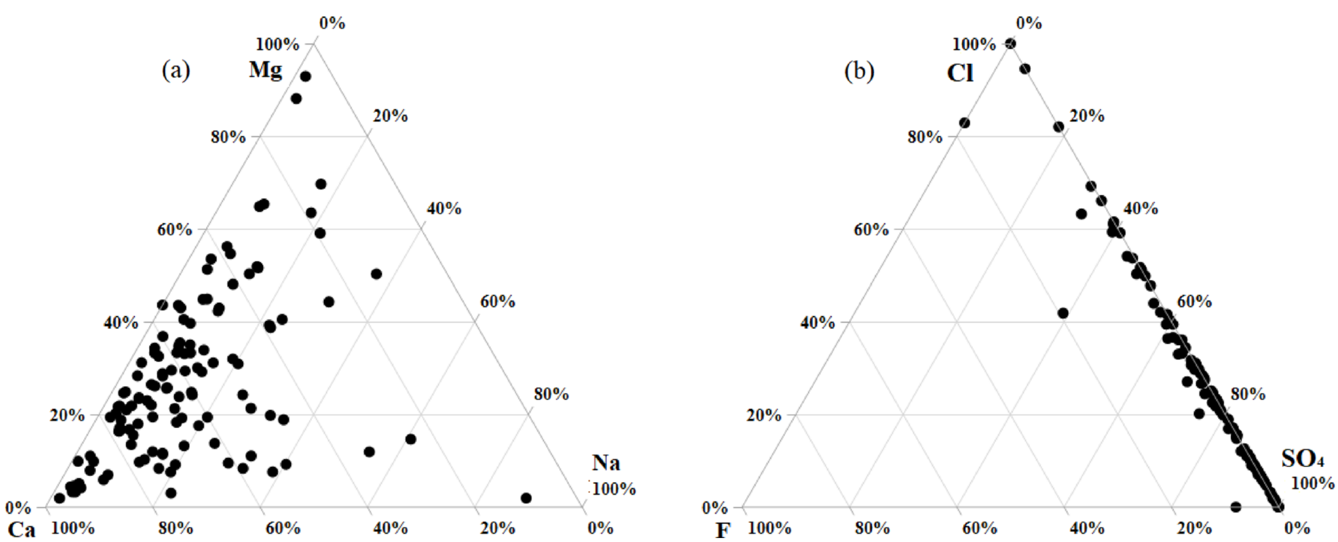


**Fig. 6.** Distribution of sampling sites among three major cations (a) ($Na^+$, $Mg^{2+}$, $Ca^{2+}$) and (b) anions ($F^-$, $SO_4^{2-}$, $Cl^-$). Water quality measurements indicated low (very low $Na^+$, low $Mg^{2+}$, and high $Ca^{2+}$) anions and relatively low cation (not significant $F^-$, very low $Cl^-$, and low $SO_4^{2-}$) values.

is probably because most species found in here were reported from relatively low to medium temperature ranges.

Three of the four most common species, except *P. olivaceus*, were generally found in waters with high (60–90%) $Ca^{2+}$ levels (Fig. 3) when $Ca^{2+}$ –range of *P. olivaceus* was relatively wider (30–90%) than others (Fig. 6). Calcium in the host waters is one of the most essential elements for ostracods because it is the main source of $Ca^{2+}$ in the carapace

(Kesling, 1951; Turpen and Angell, 1971; Rosenfeld, 1982; Majoran *et al.*, 1999). Thereby, such a demand on $Ca^{2+}$ may take attention of the species in the waters with amount of $Ca^{2+}$ enough to produce $CaCO_3$ in carapace. In contrast, since $Mg^{2+}$ is relatively low in freshwater ostracod carapaces (Wansard *et al.*, 1999; Palacios-Fest and Dettman, 2001), finding species from the water bodies with low $Mg^{2+}$ concentrations may not be surprising at all. According to Meisch (2000), *H. incongruens* was ecologically characterized as titanoeuryplastic (occurring indifferently from 0 to >72 mg $Ca^{2+}$ $L^{-1}$ ranges), mesothermophilic (preferring or tending to be found between permanent and temporarily cold waters) (and/or medium temperature ranges), and oligohalophilic (?mesohalophilic) (±0.5 to ±5‰ total salt contents) while *H. salina* was characterized as thermoeuryplastic (with wide temperature ranges), mesopolytitanophilic (mainly occurring from 18 to >72 mg $Ca^{2+}$ $L^{-1}$), and mesohalophilic (± 5 to ±18‰ total salt contents). On the other hand, *P. olivaceus* was ecologically characterized as oligothermophilic (preferring cold waters, mainly found in cold waters), euryplastic for substrate (Nüchterlein, 1969) when *I. bradyi* was classified as oligothermophilic and rheoeuryplastic (found in both stagnant and flowing waters) by Nüchterlein (1969) whereas as polythermophilic (preferring "warm" waters, mainly found in "warm" waters), mesorheophilic and meso- to polytitanophilic (mainly occurring at >72 mg $Ca^{2+}$ $L^{-1}$) by Vesper (1975).

Some of these ecological characteristics do not match with our findings. For example, we found *H. incongruens* in a variety of habitats (Tab. 2) where water temperature was cold (12.9 °C) to cool (21 °C). However, the species has already been reported from waters with much higher temperature ranges up to 32 °C (Külköylüoğlu *et al.*, 2012), or even higher than 37 °C (Akdemir, pers. obs.), referring to polythermophilic characteristics of the species that can tolerate wide temperature ranges without showing any specific tendency for cold waters. Additionally, occurrence of *H. incongruens* in deeper parts of the lakes may not be common (Akdemir and Külköylüoğlu, 2014; Akdemir *et al.*, 2016), pointing that shallow water bodies may be better places for the species. Similarly, $Ca^{2+}$-range of the species was found up to 119 $Ca^{2+}$ ppm supporting "titanoeuryplastic" characteristics (this study). In terms of salinity contents, the species was reported from oligohalophilic or slightly higher ranges during the present study but Mezquita *et al.* (1999a, b) reported the species in the waters of Iberian Peninsula where electrical conductivity (referring to salinity) was reaching about 3320 μS $cm^{-1}$, indicating eu-polyhaline ranges (see details in Külköylüoğlu, 2013). Another species of the same genus, *H. salina* showed wider ranges of temperature (up to 22 °C) and salinity (EC = 2281 μS $cm^{-1}$) than *H. incongruens*, supporting earlier ecological characterization stated by Meisch (2000). Supporting evidence was also provided by Mischke *et al.* (2012) who reported that *H. salina* in hyperarid habitats did not display particular habitat preferences for the waters dominated by anion and/or cations in Israel and Jordan where the species did show tolerances to high salinity ranges.

The next two most common species, *P. olivaceus* and *I. bradyi* were actually reported with higher temperature and salinity ranges than suggested before. Of which, *P. olivaceus* was reported from 17–107 mg $Ca^{2+}$ ranges (titanoeuryplastic, but mainly polytitanophilic) while *I. bradyi* was found between 25–103 mg $Ca^{2+}$ ranges (titanoeuryplastic). These results also correspond with higher tolerance ranges of these species than most other species indicated above. According to Meisch (2000), species with wide ecological tolerance might probably be due to their "general purpose genotype" characteristics. Although this approach deserves considerable credits, it refers species occurrences in different (or similar) habitats and environmental conditions. Besides, due to lack studies (*e.g.*, see Havel *et al.*, 1990; Yin *et al.*, 1999) on the relationship between genotype and environmental factors, we need more solid evidences to support this view. Both laboratory (Yu *et al.*, 2009, Külköylüoğlu pers. obs.) and field studies (Schneider *et al.*, 2016; Külköylüoğlu *et al.*, 2017; Yavuzatmaca *et al.*, 2017b) showed that most (if not all) ostracod species do have higher ecological tolerance ranges than what we know previously. Hence, at the moment, ecological knowledge about individual species tolerance (and optimum estimates) ranges is not promising to come up with a general conclusion. It is however true that a few species may have relatively narrow distributional ranges in particular habitats where ecological conditions are suitable for the species.

Like the common species hitherto discussed, some other species with low frequency of occurrences can also be considered for discussion. For example, *L. inopinata* has generally been encountered from lakes, ponds, swamps and ditches but also known from flowing waters. Baltanás and Geiger (1998) argued that habitat (referring to environmental conditions) preferences of this species coincided with genetically similar individuals whose body size would be larger in stagnant (lentic) or less fluctuating waters than those found in oligo-mesohaline and fluctuating (lotic) waters. Yin *et al.* (2001) working on living *L. inopinata* specimens collected from more than 50 lakes and small water bodies in the Tibetan Plateau reported that $Mg^{2+}$ was an important role on determining the body length of the species which increased until $Mg^{2+}$ level reached up to 0.12 g $L^{-1}$. In contrast, when the level was higher than 0.12 g $L^{-1}$, body size was not increased. We reported this species from five different sites of lakes, ponds and reservoirs during this study within a wide EC ranging from 367 to 1572 μS $cm^{-1}$ where percent $Ca^{2+}$ concentration was relatively lower than $Mg^{2+}$. In general, our findings agree with the ecological characterization of Meisch (2000) as that species is polythermophilic, titanoeuryplastic (occurs also in waters with poor $Ca^{2+}$ concentrations) and mesohalophilic. In our case, since the species were collected from shallow littoral zones of those lentic water bodies, we did not observe such size difference among adults. Additionally, it is also important to mention that *L. inopinata* seems to be found in large bodied natural habitats more than artificial habitats. For example, while 15 species were found from several different habitat types including troughs, *L. inopinata* was among those eight other species not found from troughs. The species was clustered together with *P. kraepelini* in group III of UPGMA dendrogram. Like *L. inopinata*, *P. kraepelini* is also known as a typical lake form (or lake-like lentic habitats). Therefore, finding these two species in the same clustering group is not surprising, and may suggest their common habitat preferences. Although CCA diagram (Fig. 5) exhibited that *L. inopinata* was located nearby the arrow of redox potential (SHE) along with *P. fontinalis*, these two species were never seen together in any of the sampling sites. Indeed, the latter species was only found from troughs during our study. Meisch (2000) stated that *P. fontinalis* was more strongly related to permanently cold waters than its conspecific *P. olivaceus* discussed above. Ecologically, *P. fontinalis* was defined as coldstenothermal, polyrheophilic, polytitanophilic (Nüchterlein, 1969), and stygophilic (Meisch, 2000). Akdemir and Külköylüoğlu (in review) provided supportive evidence about these characteristics, underlining that *P. fontinalis* had low tolerance values for dissolved oxygen in the stream, pond and spring waters of Hatay region in Turkey.

According to CCA diagram (Fig. 5), the three least common species (*P. zenkeri*, *P. villosa* and *P. variegata*) were located on the opposite sites of the variables (arrows), suggesting that these variables may not be strongly effective on these species or there was a negative correlation. One of the most morphologically shared characteristics of these species is the presence of swimming setae on second antenna (except *P. zenkeri* whose setae are medium size). Such characters may help species moving among the aquatic plants in slow flowing and/or shallow water bodies. Ecologically, these species also illustrate common characteristics as being oligothermophilic, mesorheophilic and polytitanophilic while *P. variegata* may have higher tolerance ranges than the other two species. We found the third species of the genus *Potamocypris*, *P. fulva*, located far away from the center of CCA in opposite to the arrows. Unlike others, *P. fulva* showed relatively low occurrence frequencies from trough and streams. The species was previously reported from caves as well (Meisch, 2000). Based on these reports, one may consider that species would probably prefer a few types of habitats with limited distribution. The species did not show high levels of ecological tolerances (Tab. 3) to different environmental variables. Due to lack of ecological knowledge, a general characterization about its habitat preferences may not be outlined at the moment; however, our results are in accordance with those of previous studies (Meisch, 2000; Külköylüoğlu, 2013; Uçak *et al.*, 2014; Yavuzatmaca *et al.*, 2017a, b) about ecological characteristics of this species.

Overall, results of this study clearly illustrate the fact that we still have very limited ecological knowledge about species habitat preferences. Also, we are aware of the fact that our sampling was conducted within a couple of days which may limit to make general conclusions about species' habitat preferences. However, our results strongly suggest that rare and/or endemic ostracods would probably provide better explanations about habitat requirements of ostracods. Thus, detailed and extensive field and laboratory studies on ecology of these species can answer several different questions about the habitat preferences

*Acknowledgements.* We would like to thank Dr. Randy Gibson (Texas Aquatic Resources Center, U. S. Fish and Wildlife Service) for his suggestions and help on the final part of the manuscript. Also, we would like to thank Ozan Yılmaz and Meriç Tanyeri for their help during sampling. Thanks are given to Drs. Nusret KARAKAYA (Abant İzzet Baysal University) for his help on major ion analyses, and Benjamin F. Schwartz (Texas State University) for him to provide us permission to use JMP® Pro 13.0.0 (64-bit) program. This study was supported by TÜBİTAK (Project no. 213O172).

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**Cite this article as**: Külköylüoğlu O, Yavuzatmaca M, Çelen E, Akdemir D, Dalkıran N. 2018. Ecological classification of the freshwater Ostracoda (Crustacea) based on physicochemical properties of waters and habitat preferences. *Ann. Limnol. - Int. J. Lim.* 54: 26