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SHORT REPORT

Na, K, Ca and Mg of intertidal caprellids (Crustacea: Amphipoda)

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Abstract

Sodium, potassium, calcium and magnesium contents were measured in the most common caprellid species inhabiting intertidal ecosystems of southern Spain (*Caprella acanthifera*, *C. danilevskii*, *C. dilatata*, *C. equilibra*, *C. grandimana*, *C. hirsuta*, *C. liparotensis* and *C. penantis*). Five gammarid species (*Ampithoe* sp., *Apherusa* sp., *Hyale perieri*, *H. schmidtii* and *Jassa marmorata*), three isopods (*Dynamene edwardsi*, *Idotea chelipes* and *Ischyromene lacazei*) and the tanid *Tanais dulongi* were also collected for comparison. Univariate and multivariate analyses showed that concentrations of Na, K and Mg were significantly higher in caprellids than in the remaining peracarids, probably due to different activity patterns associated with different feeding strategies. On the other hand, isopods and tanaids showed higher Ca concentrations than caprellids and gammarids, probably due to stronger cuticles in more robust bodies. Caprellid females showed higher concentrations of Ca than males and lower Na and K concentrations, related to reproductive purposes (e.g. egg composition) and possible differences in activity patterns during the clinging behaviour. This work represents the first study of cations in caprellids.

Key words: *Sodium, potassium, calcium, magnesium, peracaridan crustaceans, southern Spain*

Introduction

Caprellids form an important trophic link between primary producers and higher trophic levels. They are an important, and in some instances the dominant, natural dietary component of many fishes (Woods 2009). Recently, several studies have shown the importance of caprellids as bioindicators of physico-chemical parameters (Guerra-García & García-Gómez 2001), as a monitoring tool for butyltin residue changes over small spatial and temporal scales (see Takeuchi et al. 2001; Ohji et al. 2002; Aono & Takeuchi 2008) and as bioindicators of trace metal contamination in intertidal ecosystems (Guerra-García et al. 2009a). Furthermore, caprellid amphipods are an overlooked resource for the aquaculture of marine finfish and they could potentially be excellent in integrated co-culture and intensive culture (Woods 2009).

In spite of this increasing interest of using caprellids in applied approaches, there is a lack of basic studies exploring the concentration of the main elements in the bodies of this group. In fact, to our knowledge, this is the first study dealing with cation concentrations in caprellids.

Crustaceans exhibit almost all possible patterns of osmotic regulation (Péqueux 1995). Typical concentrations of Na^{+}, K^{+}, Mg^{2+} and Ca^{2+} in crustacean haemolymph vary greatly from species to species (Burton 1967, 1995), but usually have a general resemblance to seawater (Burton 1967). Apart from some general literature dealing with ionic regulation in general crustaceans (see Burton 1986, 1995; Morritt & Spicer 1993; Lucu & Towle 2003), most of the physiological studies in amphipods have been focused on amphipod members of the family Gammaridae (Lockwood 1970; Sutcliffe 1971a,b; Dorgelo 1977) and Talitridae (Morrit & Spicer 1998; Calosi et al. 2007). In spite of the abundant information provided on the composition of body fluids in a wide variety of crustaceans (Péqueux 1995), the knowledge of absolute concentrations of Na, K, Ca and Mg ions in peracaridan crustaceans in general is very scarce, and caprellids are totally unstudied in this

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sense. For this reason, the objective of the present study was to explore the cation concentrations in caprellids and to compare it with those of other related groups of peracaridan crustaceans, including gammarids, isopods and tanaids.

Material and methods

For this study, a total of 18 rocky shore stations were selected along the Atlantic and Mediterranean coasts of southern Spain (Figure 1). Eight caprellid species were selected for the study (*Caprella acanthifera* Leach, 1814, *Caprella danilevskii* Czerniavskii, 1868, *Caprella. dilatata* Krøyer, 1843, *Caprella equilibra* Say, 1818, *Caprella grandimana* (Mayer, 1882), *Caprella hirsuta* Mayer, 1890, *Caprella liparotensis* Haller, 1879 and *C. penantis* Leach, 1814). Besides the caprellid species, several peracaridan crustaceans were also selected (the gammarids *Ampithoe* sp, *Apherusa* sp., *Hyale perieri* (Lucas, 1849), *Hyale schmidtii* (Heller, 1866) and *Jassa marmorata* Holmes, 1903, the isopods *Dynamene edwardsi* (Lucas, 1849), *Idotea chelipes* (Pallas, 1766) and *Ischyromene lacazei* Racovitza, 1908 and the tanaid *Tanais dulongii* Audouin, 1826. The selected species of amphipods (caprellids and gammarids), isopods and tanaids are among the dominant taxa in intertidal communities of the Strait of Gibraltar (Guerra-García et al. 2009b). All the samples were collected from the intertidal area during May and June 2008. The hydroid *Tubularia crocea*, the bryozoan *Bugula neritina* and the algae *Asparagopsis*

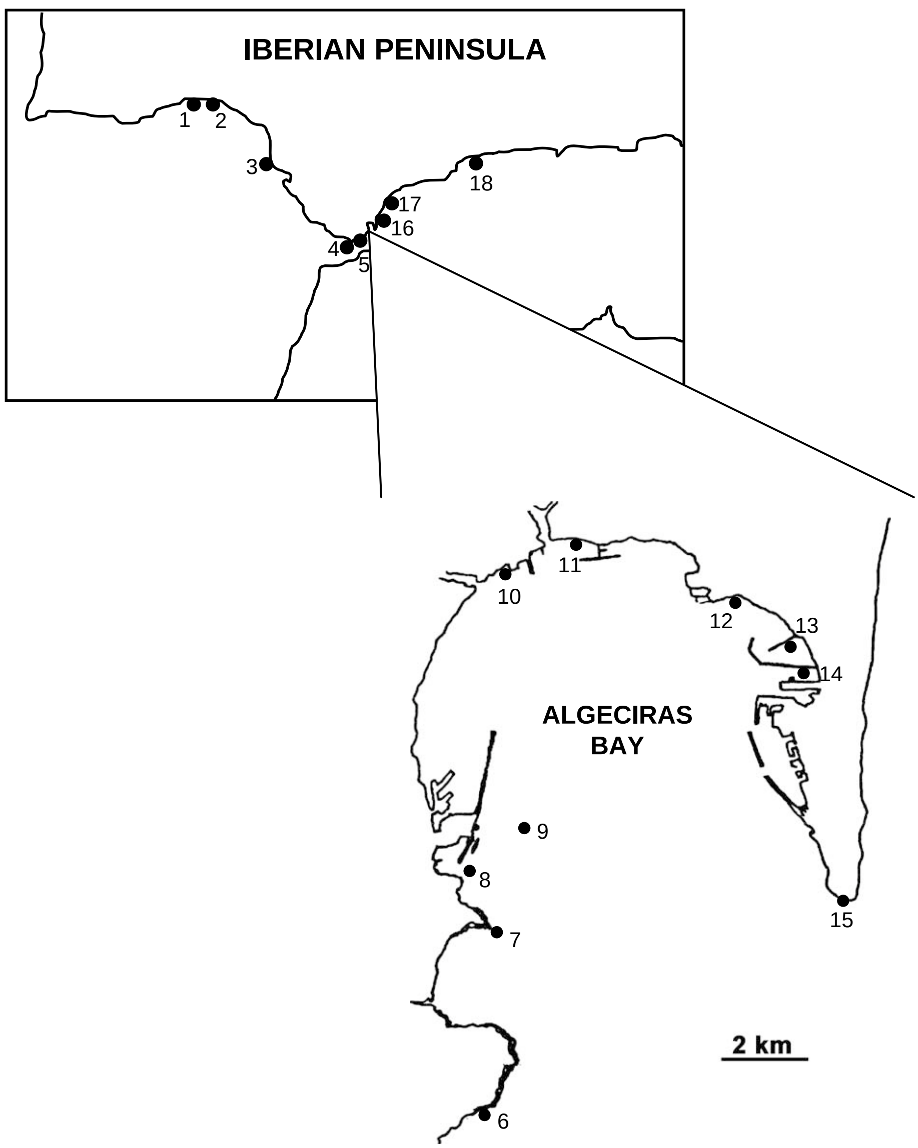

Figure 1. Location of sampling sites. 1: El Portil, 2: Ría de Huelva, 3: Sanlúcar de Barrameda, 4: Isla de Tarifa, 5: La Caleta, 6: Punta Carnero, 7: San García, 8: El Saladillo, 9: Buoy, 10: Palmones, 11: Guadarranque, 12: La Línea, 13: San Felipe harbour, 14: Gibraltar 1, 15: Gibraltar 2 (Punta Europa), 16: La Atunara, 17: Torreguadiaro, 18: Málaga.

armata, *Corallina elongata*, *Gelidium latifolium*, *Ulva* sp., *Chaetomorpha* sp., *Jania rubens* and *Stypocaulum scoparium* were collected. A first preliminary sorting of crustaceans were carried out '*in situ*'; the material was transported to the laboratory in plastic containers, sorted by species and then killed by freezing. Taking into account that at least 100 mg of dry weight are necessary for each sample to properly address the chemical protocol, adult specimens of the same species were pooled together. For caprellids, males and females were kept in separate vials for further comparison. All peracaridan samples were dried at 30°C until constant weight and then finely ground. The dry, powdered solid tissue was accurately weighed in a dry, pre-cleaned Teflon digestion vessel. Two millilitres of HNO_3, 1 ml of HCl and 3 ml of H_2O_2 were added to each vessel. The vessels were sealed and placed in a microwave chamber Anton Paar (Multiwave 3000 with a rotating 16-position sample carousel) at 240°C for 20 min with a maximum pressure of 40 bar. After digestion, the solution was brought to 25 ml volume with deionized waters. Analytical determinations were performed using Inductively Coupled Plasma–Optical Emission Spectrophotometer (ICP-OES Horiba Jobin-Yvon, Ultima 2). Patterns used for ICP measures included Merck ICP multi-element calibration standard solution IV (HC612727) and XII (OC461429). The recovery rate ranged between 85 and 115%. Possible differences of Na, K, Ca and Mg contents among caprellids, gammarids, isopods and tanaids were tested with one-way ANOVA, after verifying normality using the Kolmogorov–Smirnov test, and the homogeneity of variances using the Levene test. For caprellids, ANOVA analyses were also conducted to explore differences between males and females. A Principal Component Analysis (PCA) was used for the ordination of peracaridan species based on Na, K, Ca and Mg contents. Univariate analyses were conducted with the SPSS v.14 and multivariate analyses were carried out using the PC-ORD program (McCune & Mefford 1997).

Results and discussion

Values of Na, K, Ca and Mg measured in the 17 peracaridan species are included in Table I. Caprellids showed a significantly higher concentration of Na, K and Mg than the other three groups (gammarids, isopods and tanaids). On the other hand, isopods and tanaids showed higher concentrations of Ca than amphipods (caprellids and gammarids) (Figure 2). The first axis of the PCA (Figure 3) absorbed 44.9% of the total variance and correlated significantly with Na, K and Ca, while the second axis absorbed 37.2% of the variance and correlated with Mg. The Mg contents clearly separated caprellids from other peracarids along axis 2. Isopods and tanaids were separated from caprellids and gammarids mainly by higher concentrations of Ca and lower concentrations of Na and K. Na and K values were highly correlated ($r = 0.73$, $p < 0.01$). These two

Table I. Range of concentrations (mg/g) of Na, K, Ca and Mg in the species analysed. Stations from which species were collected are also indicated (see Figure 1).

	Stations	Na	K	Ca	Mg
CRUSTACEANS:					
Caprellids					
Caprella acanthifera	4, 7, 17	6.8–57.2	8.9–11.8	21.5–77.6	14.3–15.9
C. danilevskii	5	35.4–67.8	5.9–8.3	45.1–55.9	12.7–14.8
C. dilatata	1	48.6–49.2	13.9–14.6	38.7–58.9	8.5–8.9
C. equilibra	2, 9, 13	3.7–56.7	0.5–15.2	5.4–75.2	1.1–12.4
C. grandimana	4, 8, 15, 17	37.7–59.4	4.5–9.7	48.5–113.9	14.9–18.9
C. hirsuta	18	47.7–112.1	5.8–8.1	49.7–75.3	19.6–23.9
C. liparotensis	12, 14	41.6–63.9	5.8–18.1	44.5–59.4	9.8–14.6
C. penantis	4–8, 17	27.7–382.8	3.8–76.6	32.3–69.5	12.4–66.4
Gammarids					
Ampithoe sp.	3	30.7	12.6	77.8	9.9
Apherusa sp.	1	13.4	31.2	93.6	7.9
Hyale perieri	4	43.1	14.8	60.3	7.9
H. schmidtii	4–8, 10–12, 15–18	5.4–78.5	0.7–11.7	56.3–121.4	8.2–14.9
Jassa marmorata	1, 2, 13	40.5–54.1	9.3–13.2	67.0–94.8	12.1–12.3
Isopods					
Dynamene edwardsi	4, 13	25.9–34.3	4.4–11.2	133.2–549.5	13.9–14.6
Idotea chelipes	5, 17	22.1–44.3	5.9–8.7	85.0–122.2	11.5–14.7
Ischyromene lacazei	8, 16	23.1–24.8	2.9–8.8	38.6–137.1	7.1–13.2
Tanaid					
Tanais dulongii	11, 17, 18	20.5–43.9	5.3–10.9	91.5–153.7	10.9–15.2

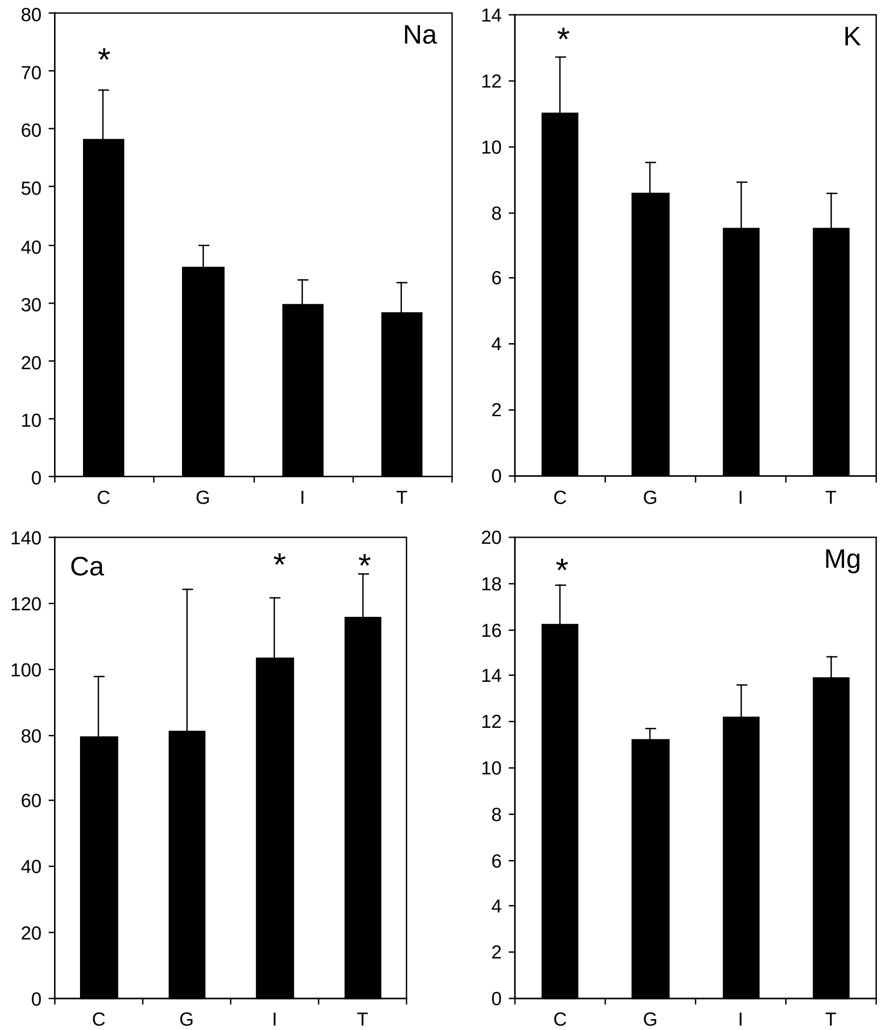

Figure 2. Mean values in mg/g (±SD) of Na, K, Ca and Mg concentrations in caprellids (C, $n=8$), gammarids (G, $n=5$), isopods (I, $n=3$) and tanaids (T, $n=1$). Significant differences at $*p<0.05$.

elements are associated with the sodium pump, or $Na^{+}+K^{+}$-ATPase, which provides at least part of the driving force for transepithelial movement of monovalent ions across the gills and other transporting tissues in many aquatic animals including the Crustacea (Lucu & Towle 2003). The significantly higher concentrations of Na and K in caprellids than in other peracarids could indicate a higher number of sodium pumps and could be associated with a higher activity: caprellids actively move their antennae and gnathopods for filter feeding (see Guerra-García & Tierno de Figueroa 2009). However, we could think that caprellids, being relatively sedentary, are less active than most gammarid amphipods, so we should look for other factors to explain higher concentrations of Na and K. There is a general trend of higher compartmentalization in more evolved groups, so caprellids could show a higher compartmentalization with higher membrane surface and, consequently, higher numbers of $Na^{+}+K^{+}$-ATPases. Anyway, further ecophysiological studies should be conducted in the near future to explain these clear differences in concentrations of Na and K in caprellids and other peracaridans. On the other hand, Mg showed also a high correlation with Na ($r=0.4$, $p<0.05$), K ($r=0.7$, $p<0.01$) and Ca ($r=0.6$, $p<0.05$). In Crustacea, generally, the average concentration of K correlates with Na and Mg (Burton 1995). Mg plays an important role as a co-factor in many enzyme systems, especially those involved in the transfer of phosphate groups (Skou 1960; Morritt & Spicer 1993). In the present study, Mg concentrations were also significantly higher in caprellids than in other groups and future ecophysiological studies are also necessary to properly explore these differences.

Ca is an essential component of the crustacean exoskeleton, activator for several key enzymes, directly involved in muscle contraction. The two main inorganic anions of crustacean exoskeleton are carbonate and phosphate, with calcium as the main

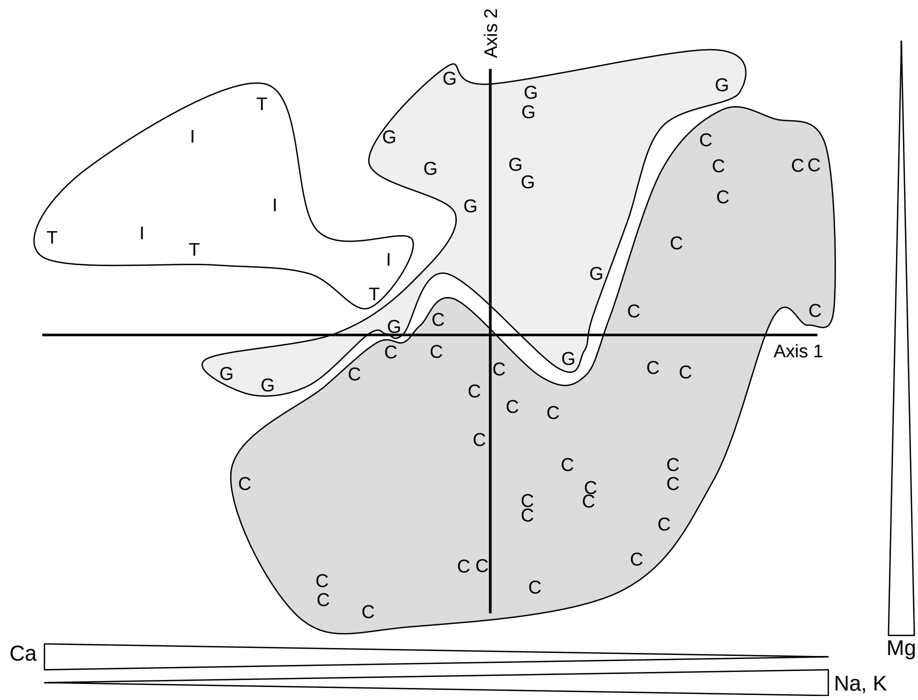

Figure 3. PCA analysis based on concentrations of Na, K, Ca and Mg in all the studied samples of the 17 peracaridan species considered. C: caprellids, G: gammarids, I: isopods and T: tanaids.

cation of both. In the present study we measured significantly higher concentrations of Ca in isopods and tanaids than in amphipods (see Figures 2 and 3). The cuticle of isopods and tanaids is usually stronger than in caprellids and gammarids; the bodies of isopods and tanaids are more robust so we could attribute these differences to a higher contribution of exoskeleton to the total animal body, explaining the higher Ca concentrations measured for these two groups.

For caprellids, females showed higher concentrations of Ca than males, while concentrations of Na and K were higher in males. These sexual differences could be related with reproduction (e.g. egg compositions) or even with different behavioural activity patterns of male and females. Sardá & Cros (1984)

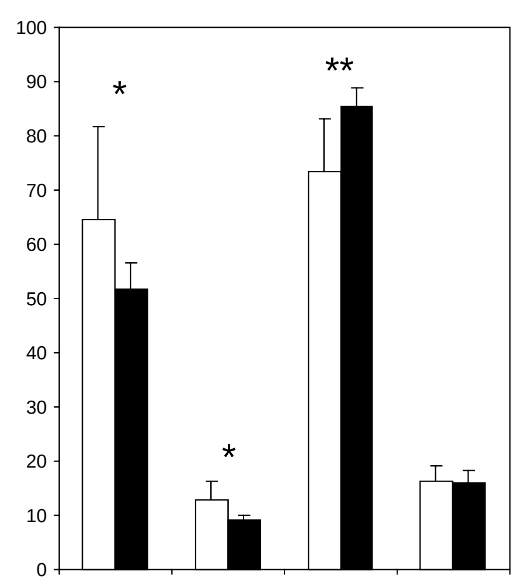

Figure 4. Mean values in mg/g (±SD) of Na, K, Ca and Mg concentrations in male (white bars) and female (black bars) caprellids. ** Significant differences at $p < 0.01$.

studied the calcium and magnesium metabolism during the moult in the decapod *Nephrops norvegicus* and found an increase of Ca and Mg concentrations after the moult, so differences between sexes could be also explained by different moulting patterns.

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