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Differences in ion regulation in the sea urchins *Lytechinus variegatus* and *Arbacia lixula* (Echinodermata: Echinoidea)

Denilton Vidolin, Ivonete A. Santos-Gouvea and Carolina A. Freire*

Departamento de Fisiologia, Setor de Ciências Biológicas, Universidade Federal do Paraná,
Centro Politécnico, Curitiba, PR 81531-990 Brazil.
*Corresponding author, e-mail: cafreire@ufpr.br

The regular sea urchin *Lytechinus variegatus*, a species previously reported from areas of reduced salinities, and *Arbacia lixula*, a species unreported from diluted waters, were submitted to seawater dilution or seawater dilution in magnesium-supplemented waters. Seawater (35 psu) was either proportionally diluted with filtered dechlorinated tap water (30 psu, 25 psu), or diluted and supplemented with magnesium as $MgCl_2$ (30+Mg, 25+Mg), up to full-strength seawater Mg^{2+} levels (35 psu, ~54 mM Mg^{2+}). Magnesium supplementation was intended to verify the interfering effect of magnesium on osmo-ionic concentrations of the coelomic fluid (CF) of two ecologically distinct species of sea urchins. After 6 h in control (35 psu) or experimental seawater, CF samples were withdrawn by puncturing through the peristomial membrane. Coelomic fluid osmolality ([Osm]), and concentrations of ($[Na^+]$), ($[Cl^-]$), ($[Mg^{2+}]$) and ($[K^+]$) were measured for both species. Under all conditions, *L. variegatus* displayed higher CF osmolality, $[Na^+]$, and $[K^+]$ values than the water (and *A. lixula*). Comparatively, *L. variegatus* is designated as a 'hyper-conformer', while *A. lixula* is an 'iso-conformer'. The CF $[Mg^{2+}]$ showed no evidence of being controlled by either species. Mg^{2+} supplementation in diluted seawater affected Mg^{2+} and Cl^- levels only. Na^+ appears to be taken up actively by *L. variegatus*, rendering its CF mostly hyper-ionic for Na^+ (and hyperosmotic) relative to external seawater. The different gradients observed with the different ions suggest selective permeabilities or ion regulation by *L. variegatus*.

INTRODUCTION

Echinoderms strictly inhabit the ocean, and are normally considered to be stenohaline, osmoconformers, isosmotic, and mostly isoionic animals. Most echinoderms are unable to either tolerate large variations in seawater salinity, or to withhold significant osmotic/ionic gradients with respect to ambient seawater (Roller & Stickle, 1985; Diehl, 1986; Roller & Stickle, 1993; Bishop et al., 1994; Roller & Stickle, 1994). Echinoderms do not possess specific osmoregulatory or excretory organs, and their integument is supposed to be very permeable to water and ions (Stickle & Ahokas, 1974; Roller & Stickle, 1993).

However, these animals are frequently found in tide pools or in estuarine areas, where they are subjected to salinity reduction (Ernest & Blake, 1981). In tidal pools during low tide, they may be aerially exposed, thus facing desiccation, exposed to increased salinity due to evaporation, or decreased salinity due to rain precipitation (Roller & Stickle, 1994), or ice melting (Stickle & Ahokas, 1974). Echinoderm populations are known to tolerate some salinity reduction (Stickle & Diehl, 1987; Foglieta & Herrera, 1996); for example, the sea-cucumber *Eupentacta quinquesemita* and the sea urchin *Strongylocentrotus droebachiensis* have a 30-day survival limit in 12–13 psu water (Sabourin & Stickle, 1981), regarded as a fair tolerance for 'stenohaline invertebrates.' Different degrees of eury/steno-halinity have been demonstrated for echinoderms, both as larvae and adults (Roller & Stickle, 1985, 1993, 1994), and an extensive list of tolerances has been provided in the review by Stickle & Diehl (1987). Conversely, mass mortality events of the echinoids *Lytechinus variegatus* have been attributed to reduced salinity (Lawrence, 1975; Watts et al., 2001). Different populations of the same species display different degrees of euryhalinity under different experimental protocols. For example, in Florida, Lawrence (1975) reported that *L. variegatus* can tolerate 23 psu seawater for five days, while Bishop et al. (1994) noted that they could tolerate 20 psu for 32 d.

These observations suggest that echinoids possess an osmoregulatory mechanism, perhaps incorporating permeability control and cell volume regulatory means (Lange, 1964; Diehl & Lawrence, 1984, 1985; Diehl, 1986; Stickle & Diehl, 1987; Foglieta & Herrera, 1996). Over the last 40 years studies have revealed differences in the ionic composition of the largest extracellular compartment, the perivisceral coelom, and the external seawater. Essential osmoconformation does not necessarily equate to the lack of a gradient and ionic conformation or iso-ionic levels for all ions, and their body fluids do not necessarily display identical solute composition as the surrounding seawater. Transepithelial ionic gradients indicating selective transport of ions in echinoderms have been ascribed to: (1) the uptake of nutrients from the gut to the coelomic fluid (gradient of Cl^- in *L. variegatus*, Bishop et al., 1994); and (2) the establishment of an osmotic gradient (gradient of K^+) to ensure the necessary turgor of ambulacral feet as in *Asterias forbesi* (Prusch, 1977). Still, another major role for ionic regulation in echinoderms has been related to the effect of specific ions on the properties of their connective tissue. This requirement may have prevented the invasion of dilute seawater by echinoderms (Eylers, 1982). However, although the apparent regulation of ion concentrations in the coelomic fluid has been vastly reported for some echinoderms, it has not been incisively interpreted as regulation arising from specific active trans-epithelial transport (Diehl, 1986).

Magnesium (Mg^{2+}) is an important co-factor for a vast number of enzymes, including those involved in the transfer of phosphate groups, the regulation of G-proteins, and the stabilization of the cytoskeleton, ion channels, and cell membranes, etc. (Beyenbach, 1990). Mg^{2+} is also an important factor affecting the viscosity of echinoderm tissues (Hidaka, 1982; Motokawa, 1994). The present work was conducted to test the working hypothesis that some sea urchins are capable of ionic regulation of their coelomic fluid, and that the imbalance of a physiologically relevant ion (Mg^{2+}), would disturb the ionic equilibria if the coelomic wall epithelium would not be fully and equally permeable to all ions (Na^+, Cl^-, Mg^{2+}, K^+). Equal permeability might be expected in sea urchins, if they are truly osmotic- and ionic-conformer invertebrates.

The investigation aimed at widening the knowledge on ionic regulation in echinoderms, comparing the pattern of response between the larger *Lytechinus variegatus*, and the smaller *Arbacia lixula*. *Lytechinus variegatus* is a shallow water species (Roller & Stickle, 1993; Böttger et al., 2001) that displays positive phototaxis (Sharp & Gray, 1962), and is occasionally found in areas of reduced salinities (25–30 psu, Roller & Stickle, 1993; Tavares & Borzone, personal communication). *Lytechinus variegatus* has been reported as stenohaline when compared to other echinoids (Roller & Stickle, 1993; Watts et al., 2001). *Arbacia lixula* is restricted to more stable environments (3–8 m deep) of full strength seawater (Sánchez-Jérez et al., 2001), its congener *A. punctulata* is negatively phototactic (Sharp & Gray, 1962). Although *L. variegatus* has been the focus of several studies (Roller & Stickle, 1993; Böttger et al., 2001; Watts et al., 2001), the only study comparing the physiology of both species reported that the adults of *L. variegatus* were equally tolerant to seawater dilution as adults of *A. lixula*: they both survive 10 days in 20–22 psu (Petersen & Almeida, 1976). Curiously, the pluteus larva of *A. lixula* is more tolerant of seawater dilution than the larva of *L. variegatus* (Petersen & Almeida, 1976). This comparative study aims to mitigate the paucity of knowledge on these invertebrates.

MATERIALS AND METHODS

Specimens of *Lytechinus variegatus* were obtained using a drag net from Ilha dos Currais, Pontal do Paraná, Paraná, Brazil (25°33′S 48°23′W) and *Arbacia lixula* by SCUBA diving, from Ilha da Galheta, Pontal do Paraná, Paraná, (25°30′S 48°15′W). Both species were collected from depths of ~3 m, and salinity of 34 psu. Urchins were taken to the laboratory where they were held for at least one week in a stock tank containing 390 l of 35 psu seawater (SW) at 22 ± 1 °C. The tank was provided with constant aeration and biological filtration. Urchins were fed daily with *Ulva* sp. algae *ad libitum*. The same experimental protocol was used for both species. Independent experiments were conducted for each species, during the months of July and August of 1999 (for *L. variegatus*), and July and August of 2001 (for *A. lixula*). For each species four experimental 20-l aquaria were prepared. Two aquaria contained seawater diluted with filtered dechlorinated tap water to 30 psu and 25 psu, respectively. The other two experimental aquaria contained seawater diluted to the same salinities, but supplemented with enough $MgCl_2$ so as to bring their magnesium concentration ($[Mg^{2+}]$) up to the levels of $[Mg^{2+}]$ in 35 psu SW. For the 30+Mg (30 psu SW) aquarium, 27.52 g of $MgCl_2$ (135 mmoles) were added to 20 l, bringing measured $[Mg^{2+}]$ from 46 mM to 54 mM. For the 25+Mg aquarium (25 psu SW), 20 l received 59.19 g of $MgCl_2$ (291 mmoles), bringing measured $[Mg^{2+}]$ from 42.5 mM to 55 mM (Table 1). Additionally, one 20-l control aquarium was also prepared, containing full-strength 35 psu SW. Table 1 shows the expected (Prosser, 1973) and measured osmotic and ionic concentrations in the waters of the different control and experimental aquaria. The measured values are an average of the two values determined for the aquaria containing either *L. variegatus* or *A. lixula*.

Six urchins of both species were placed in each 20-l aquarium and exposed to each control or experimental treatment. Urchins were not fed during the experimental period. Coelomic fluid samples (~500 µl) were withdrawn by puncturing the peristomial membrane with a disposable insulin syringe, after six hours of exposure of the animals to either control or experimental conditions. After the withdrawal of the single coelomic fluid sample, urchins remained under observation in the same aquaria for five days, when algae were again offered every day.

Table 1. *Expected (E, from Prosser, 1973) and measured (M) values for osmolality and ions in water of control (35 psu), and experimental aquaria with diluted seawater (30 and 25 psu salinity), or diluted seawater supplemented with magnesium (30+Mg and 25+Mg), used for the two species of sea urchins,* Lytechinus variegatus *and* Arbacia lixula. *Measured values (M) are the average levels of the samples taken from the aquaria used for both species.*

	Osm		Na^+		Cl^-		Mg^{2+}		K^+	
Aquarium S psu*	E	M	E	M	E	M	E	M	E	M
35	1050	986	479	485	559	563	54	56	10	9.8
30	900	866	411	428	479	456	47	46	8.5	8.5
25	750	745	342	352	399	398	39	43	7.0	6.9
30+Mg	900	835	411	405	493	445	54	54	8.5	8.5
25+Mg	750	760	342	368	428	418	54	55	7.0	7.0

Measured parameters: osmolality ([Osm], mOsm/kg H_2O), sodium ($[Na^+]$, mM), chloride ($[Cl^-]$, mM), magnesium ($[Mg^{2+}]$, mM), and potassium ($[K^+]$, mM) concentrations. *, Measured salinity (S psu) of the water.

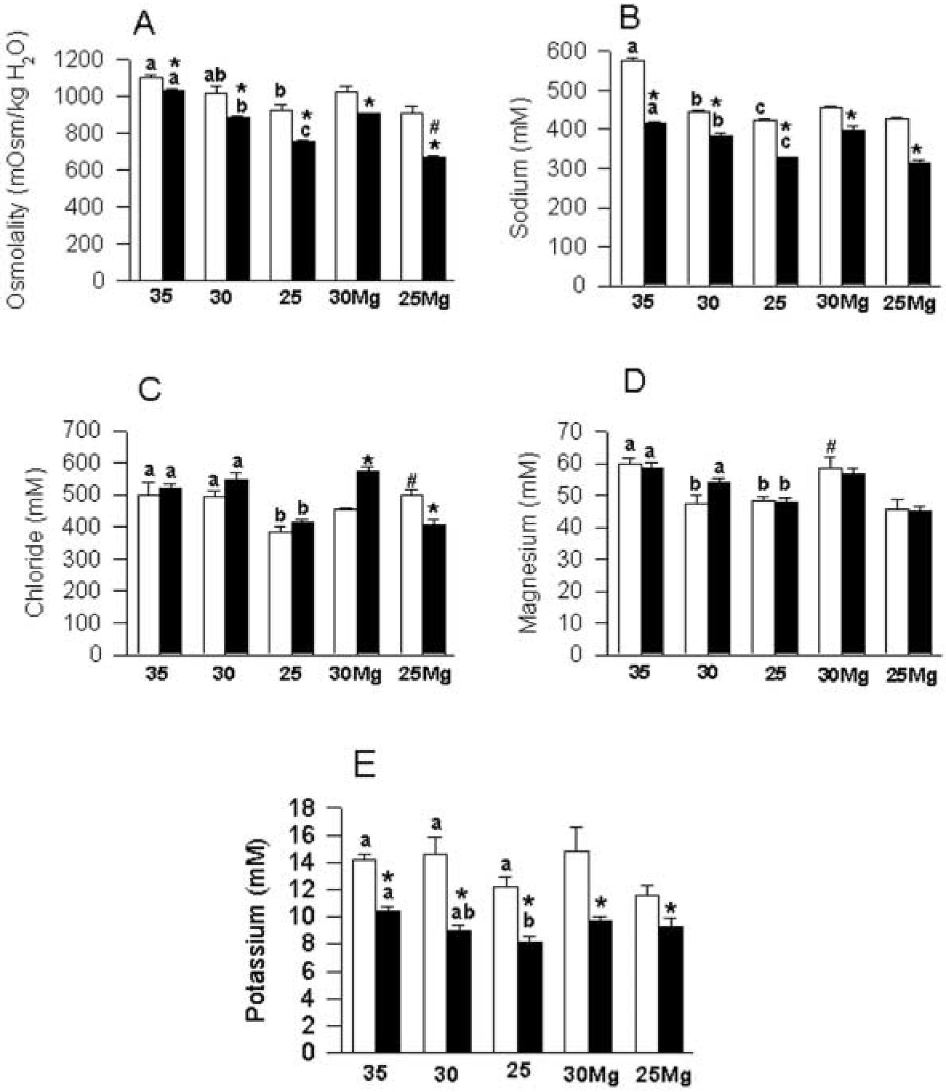

Figure 1. (A) Osmolality (mOsm/kgH_2O); (B) sodium (mM); (C) chloride (mM); (D) magnesium (mM); (E) potassium (mM) (mean ±standard error of the mean, N=6) values measured in the coelomic fluid of *Lytechinus variegatus* (white bars) and *Arbacia lixula* (black bars), submitted to the following waters: 35 psu (control), 30 psu, 25 psu, 30 psu supplemented with Mg^{2+} (30 Mg), 25 psu supplemented with Mg^{2+} (25 Mg), for 6 h. *, *A. lixula* ≠ *L. variegatus*, #, supplemented diluted seawater ≠ non-supplemented diluted seawater of same salinity. Bars that share one common letter (lower case) are not statistically different; 35 psu, 30 psu, 25 psu within the same species. Level of significance set at 0.05.

Table 2. *Gradients (differences between coelomic fluid and water), for measured parameters in the two species of sea urchins* Lytechinus variegatus *(Lv) and* Arbacia lixula *(Al), after 6 h in either control (35 psu) or experimental aquaria with diluted seawater (30 and 25 psu salinity), or diluted seawater supplemented with magnesium (30 Mg and 25 Mg).*

Aquarium	Sp.	[Osm]	[Na^+]	[Cl^-]	[Mg^{2+}]	[K^+]
35	Lv	112 ±18*	91 ±8*	−62 ±38	4 ±1.7	4.4 ±0.4*
	Al	46 ±5	68 ±4*	−41 ±12*	2.6 ±1.8	0.6 ±0.4
30	Lv	152 ±36*	30 ±5	90 ±17*	1.2 ±3	6 ±1.3*
	Al	22 ±2.1	−45 ±9	91 ±24*	8 ±1.3	0.6 ±0.5
25	Lv	182 ±3*	71 ±3*	−14 ±15	6 ±1.6	5 ±0.7*
	Al	9 ±9	−24 ±2	16 ±11	5 ±1.1	1.2 ±0.5
30 Mg	Lv	188 ±30*	50 ±5	21 ±13	2.5 ±3	6 ±1.8*
	Al	73 ±2*	−11 ±10	128 ±14*	2.7 ±1.9	1.2 ±0.3*
25 Mg	Lv	152 ±34*	61 ±4*	89 ±17*	−10 ±3*	4.6 ±0.7*
	Al	86 ±6*	−53 ±7*	−12 ±18	−10 ±1.4*	2.3 ±0.6*

Measured parameters: osmolality ([Osm], mOsm/kg H_2O), sodium ([Na^+], mM), chloride ([Cl^-], mM), magnesium ([Mg^{2+}], mM), and potassium ([K^+], mM) concentrations. *, Significant difference ($P < 0.05$) between values of coelomic fluid and measured in the water.

Samples were immediately frozen at 20°C until use for osmotic and ionic determinations. Osmolality, Na^+, Cl^-, Mg^{2+} and K^+ concentrations were assayed from the coelomic fluid and water samples. Osmolality was measured using a Wescor 5520 VAPRO (USA) vapour-pressure micro osmometer. Na^+ and K^+ concentrations were determined in duplicates using a flame photometer (Digimed DM61, Brazil). Cl^- and Mg^{2+} concentrations were assayed in duplicates through colorimetric methods, using commercially available kits (Labtest, Lagoa Santa, Brazil). Cl^- ions react with mercury thiocyanate in the presence of ferric nitrate with absorbance read at 470 nm (ULTROSPEC 2100 Pro-Amersham Pharmacia Biotech). Mg^{2+} ions react with sulphonated magon in alkaline medium, with absorbance read at 505 nm. Samples were appropriately diluted with deionized water so that absorbance would fall within the linearity range for each method.

Urchin surface area to volume ratio was estimated using six dry tests of urchins from either species, randomly chosen among the 30 urchins of each species used for the physiological experiments. Surface area (S) was estimated considering the urchins as a spheroid dome, and measuring their height h and radius r: $S=\pi(h^2+r^2)$. The volume of the urchins was estimated from water displacement inside a graduated cylinder, with the urchins tests carefully and thoroughly wrapped by saran wrap.

Confidence intervals (95%) were used for the comparison between coelomic fluid values and measured ambient seawater values, in order to identify the establishment of significant gradients. The coelomic fluid values from each species in 35, 30, and 25 psu were compared using one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test (or Kruskal–Wallis when data did not pass the normality test) to localize differences and allow conclusions of conformation and regulation. Unpaired Student's *t*-tests (or Mann–Whitney, when data did not pass the normality test) were employed for direct comparisons between the two species, and in comparing the supplemented group with the non-supplemented submitted to the same salinity. Significance level of each test was set at $P<0.05$.

RESULTS

General observations

Urchins of both species exposed to 25 psu seawater supplemented with magnesium (25+Mg) displayed loss of movement of their spines and ambulacral feet, and did not feed for three days. On the fourth day of exposure, feeding and their normal behaviour resumed. Simple seawater dilution to 30 or 25 psu, or dilution to 30 psu with magnesium led to no behavioural changes in the urchins of either species, for the whole period of observation (five days).

Osmolality

The osmolality of the coelomic fluid (CF) of *Lytechinus variegatus* was higher than that of the water at all experimental situations. In contrast, CF osmolality of *Arbacia lixula* was hyperosmotic only to ambient water osmolality for those groups of urchins exposed to salinity reduction and provided with magnesium supplementation (Table 2). Both species hyperconformed, but *L. variegatus* maintained steeper gradients with respect to the ambient water than *A. lixula*, and always displayed a higher osmolality than *A. lixula* (Figure 1A). For *L. variegatus*, only the group in 25 psu had lower osmolality than the controls in 35 psu, while for *A. lixula*, exposure to 30 psu produced lower CF values. Magnesium supplementation of the water led to reduction in the osmolality of the CF of *A. lixula* in 25 psu (Figure 1A).

Sodium

Similar to the pattern observed with the osmolality analysis, the sodium concentration ($[Na^+]$) in the CF of *L. variegatus* was higher than the $[Na^+]$ in the water, in controls (35 psu), in the group in seawater diluted down to 25 psu, and in the group 25+Mg. For *A. lixula*, only the control group had higher $[Na^+]$ in their CF when compared to the water (35 psu). *Arbacia lixula* in 25+Mg had a lower $[Na^+]$ than the water to which they had been exposed for 6 h (Table 2). Following the osmolality result, $[Na^+]$ in the CF of *A. lixula* was always lower than that of *L. variegatus*. Both species showed conformation for sodium, with values decreasing from 35 psu to 30 psu, then to 25 psu. Magnesium supplementation in the water had no effect on CF sodium for either species (Figure 1B).

Chloride

Coelomic fluid chloride displayed a more varied pattern. CF $[Cl^-]$ of *L. variegatus* was above the $[Cl^-]$ of the 30 psu and 25+Mg waters. CF $[Cl^-]$ of *A. lixula* was above that of the water in 30 psu and 30+Mg waters, but below that of the water in control seawater (35 psu) (Table 2). The two species were different with respect to CF $[Cl^-]$ only in the magnesium supplemented waters: *A. lixula* CF $[Cl^-]$ was above that of *L. variegatus* in 30+Mg, but below that of *L. variegatus* in 25+Mg. Both species maintained constant CF $[Cl^-]$ when seawater salinity was reduced down to 30 psu. Further dilution of seawater to 25 psu led to reduction in CF $[Cl^-]$. Magnesium supplementation of the 25 psu seawater led to an increase in the CF $[Cl^-]$ of *L. variegatus*, when compared to urchins kept in plain 25 psu water (Figure 1C).

Magnesium

Coelemic fluid values of $[Mg^{2+}]$ of both *L. variegatus* and *A. lixula* were lower than ambient water levels for 25+Mg seawater (Table 2). CF magnesium concentrations were always similar between the two species. After six hours in 30 psu seawater, the $[Mg^{2+}]$ of the CF of *L. variegatus* decreased with respect to the control value in 35 psu, but did not decrease further when in 25 psu. On the other hand, CF $[Mg^{2+}]$ of *A. lixula* did not decrease in 30 psu when compared to the controls in 35 psu, but decreased in 25 psu when compared to values in 35 and 30 psu. The only effect of magnesium supplementation on CF $[Mg^{2+}]$ was noted for *L. variegatus*, which displayed an increase in its CF $[Mg^{2+}]$ in 30+Mg water when compared to the value of the group in 30 psu (Figure 1D).

Potassium

Coelemic fluid $[K^+]$ of *L. variegatus* was above the $[K^+]$ of the water for all experimental conditions. In contrast, in

A. lixula only the groups in 30+Mg and 25+Mg displayed higher [K^+] in their CF than the [K^+] of the water (Table 2). [K^+] in the CF of *L. variegatus* was always higher than the [K^+] in the CF of *A. lixula*, and was stable in 35, 30, and 25 psu seawater. The [K^+] in the CF of *A. lixula* in 35 psu was maintained in 30 psu, but was reduced in 25 psu. Finally, there was no effect of magnesium supplementation on CF [K^+] of both species (Figure 1E).

Size and surface area to volume ratio

Arbacia lixula, of average smaller size than *L. variegatus*, displayed a higher surface area to volume (S/V) ratio (38.8 ±1.5 mm of test diameter, 1.15 ±0.058 cm^{-1} of S/V, N=6 urchin tests, mean standard error) than *L. variegatus* (48.6 ±1.2 mm of test diameter, 0.83 ±0.048 cm^{-1} of S/V, N=6); P=0.0016 for the S/V comparison between both species.

DISCUSSION

Echinoderms have traditionally been considered stenohaline marine invertebrates. However, the present results suggest that at least some species of sea urchins can withstand a 30% reduction in salinity from full-strength (35 psu) seawater, to 25 psu for five days. This degree of seawater dilution occurs naturally in tidal pools during heavy rainfall, although only for a few hours. Adult *Lytechinus variegatus* have been reported to tolerate salinities of 18–20 psu (Roller & Stickle, 1993; Bishop et al., 1994). Considered more euryhaline, the sea urchins *Strongylocentrotus droebachiensis* and *S. pallidus* have been exposed stepwise (2.5 psu intervals) to salinities ranging from 27.5 psu down to 10 psu, for up to four weeks (Roller & Stickle, 1994). Our results indicate that no difference in tolerance exists between *Lytechinus variegatus* and *Arbacia lixula*, as previously observed by Petersen & Almeida (1976).

Urchins (*Lytechinus variegatus* and *Arbacia lixula*) submitted to 25 psu seawater with $MgCl_2$ supplementation displayed lack of spine and ambulacral feet movement, an indication of stress. According to Ruppert & Barnes (1994), the connection of the spine with its tubercle in the test is through two rings, composed of a collagen layer and a muscular layer. Reduction in the muscular tonus of the spines would be expected and was indeed observed in animals exposed to salinity reduction with $MgCl_2$ addition, due to the muscle relaxant effect of magnesium. Still, a specific role for magnesium on this effect is not clear, as magnesium was brought up to full-strength SW levels, and the same was not observed in the urchins submitted to seawater of salinity 30 psu, supplemented with magnesium. Feeding and locomotor consequences of salinity reduction have been found by Bishop et al. (1994) and Irland et al. (1997) with *L. variegatus* submitted to seawater of 20 psu. The podia of *S. droebachiensis* lost tonus in diluted SW (approximately 43% seawater dilution, Stickle & Ahokas, 1974). Thus, it is clear that salinity alterations (here with additional ionic alteration) in the water cause physiological effects in echinoderms.

Lytechinus variegatus has always displayed coelomic fluid osmolality values above those of seawater, and above those of the coelomic fluid of *A. lixula*. This difference can possibly be accounted by the concentrations of sodium and potassium (not as much), which are also always higher in the coelomic fluid of *L. variegatus* than in the water or in the coelomic fluid of *A. lixula*. There must be a relevant anion to ensure electroneutrality of the fluid, but its identity was not revealed by this study. This anion could well be sulphate, very abundant in seawater. Curiously, planktonic organisms hypo-regulate the heavy anion sulphate in order to gain positive buoyancy. It is possible that non-sessile benthic animals utilize an opposite pathway, accumulating sulphate in their body fluids. This has not been reported in the literature and data reviewed on coelomic fluid concentrations in echinoids and asteroids did not indicate any trend towards sulphate accumulation (Stickle & Diehl, 1987). The trend of being hyper-ionic for Na^+ and possibly hypo-ionic for Cl^- (Lange, 1964) is not new for echinoderm coelomic fluid data (Stickle & Diehl, 1987), and confirmed previous data obtained for *L. variegatus* (Bishop et al., 1994). However, species variability is again noted here, as absence of Cl^- gradients (Stickle & Diehl, 1987) or even a hyper-ionic state for Cl^- (Stickle & Denoux, 1976) have both been noted for other echinoids. This species variability prevents the establishment of a clear pattern of gradients being sustained by echinoids, between their extracellular fluids and external SW (Diehl, 1986; Stickle & Diehl, 1987). Accordingly, echinoderms are normally reported to be isosmotic or slightly hyperosmotic to the external seawater (Diehl, 1986). *Lytechinus variegatus* was shown here to be hyperosmotic, unlike previous data from another population of this urchin. Bishop et al. (1994) reported *L. variegatus* from Florida to be isosmotic, despite a hyper-ionic state for Na^+.

The species difference found in the present study, with respect to the capacity to sustain gradients relative to the external water could be explained by an apparent higher body wall permeability in *A. lixula* than in *L. variegatus*, due to the smaller size of *A. lixula*. Indeed, Stickle & Ahokas (1974) and Stickle & Denoux (1976) discuss the time differences in equilibration time of coelomic fluid osmotic and ionic concentrations with respect to the body size for the urchin *Strongylocentrotus droebachiensis*, concluding that larger urchins indeed survive longer times in low salinities than urchins of smaller size.

The mean osmolality and potassium gradients established between the coelomic fluid of the urchins and the ambient seawater were compared between *L. variegatus* and *A. lixula*. These were the two parameters for which *L. variegatus* displayed significant gradients for the five experimental conditions (Table 2). The mean osmotic gradient sustained by *L. variegatus* was of 157.2 ±13.5 mOsm/kg H_2O, N=5, while that of *A. lixula* was of only 47.2 ±14.6 mOsm/kg H_2O (P=0.00056). The mean potassium gradient sustained by *L. variegatus* was of 5.2 ±0.34 mM, N=5, while that of *A. lixula* was of only 1.18 ±0.31 mM (P=0.000023). Thus, the mean gradients sustained by *L. variegatus*, osmotic and of potassium, were respectively 3.3- and 4.4-fold higher than the gradients sustained by *A. lixula* (Table 2). On the other hand, the mean surface area to volume ratio of *A. lixula* (1.15 cm^{-1}) was only 1.39-fold higher than that of *L. variegatus* (0.83 cm^{-1}). Thus, a size effect does not seem to be the case here, as the gradients established by *L. variegatus* are steeper than what could be expected from the relative surface difference. And further, all ions would be expected in theory to show the same pattern, and this definitely did not happen (Table 2).

Lytechinus variegatus remains hyperosmotic with respect to seawater, although displaying coelomic fluid dilution upon seawater dilution, in a pattern that could be described as 'hyperconformation'. *Lytechinus variegatus* may be found in estuarine environments (Ernest & Blake, 1981; Roller & Stickle, 1993; Y. Tavares & C.A. Borzone, personal communication), but the same has not been reported for *A. lixula*, a finding that may be related to the differences detected in the present study. Thus, even if echinoderms are generally conformers, species-specific differences have been demonstrated to exist, not simply related to surface/volume differences (Stickle & Ahokas, 1974; Stickle & Denoux, 1976; Roller & Stickle, 1985).

The small but significant gradient for potassium may also mean significant uptake, but would not be relevant osmotically. Gradients of potassium across different epithelia have been reported for echinoderms, and have been related to nutrient transport across the intestinal epithelium as reported for *L. variegatus* (Bishop et al., 1994), or to keep ambulacral feet of sea stars turgid (Robertson, 1949; Prush, 1977; Diehl, 1986). In the ambulacral fluid of asteroids, values of K^+ concentration 18–93% above those in SW have been reported (Diehl, 1986). Potassium gradients between the perivisceral coelomic fluid and external seawater have been demonstrated for several echinoderms (Stickle & Denoux, 1976; Diehl, 1986). Data for *L. variegatus* and *A. lixula* here are within the range reported for other echinoids (Diehl, 1986; Stickle & Diehl, 1987). The gradient of 4–6 mM K^+ noted here for *L. variegatus* is in accordance with data for other echinoids, which seem to sustain a higher gradient than other echinoderms (Diehl, 1986). On the other hand, magnesium, despite its broad physiological relevance, was not regulated by *L. variegatus* or *A. lixula*, a result also previously reported for other urchin and echinoderm species (Stickle & Diehl, 1987).

Lytechinus variegatus has thus shown results compatible with mechanisms for salt absorption, maintaining a hyper-osmotic and hyper-ionic CF for some ions, notably sodium and potassium, even in full-strength seawater. This could have evolved as an adaptation to prevent significant salt loss during the probable event of seawater dilution, either in low tide or near an estuary. At least a limited capacity of ion regulation seems to be possible, especially in echinoids that inhabit more coastal or shallow waters, where they are likely to experience periodic salinity fluctuations. Differential maintenance of ions by echinoid epithelia, with faster ion exchange in smaller urchins with higher surface area/volume ratios have been proposed (Stickle & Denoux, 1976; Diehl, 1986). These conclusions have been strengthened by the data presented in the current study.

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