



Direct relationship between osmotic and ionic conforming behavior and tissue water regulatory capacity in echinoids

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ABSTRACT

Echinoderms are considered marine osmoconforming invertebrates. However, many are intertidal or live next to estuaries, tolerating salinity changes and showing extracellular gradients to dilute seawater. Three species of echinoids – *Lytechinus variegatus*, which can occur next to estuarine areas, the rocky intertidal *Echinometra lucunter*, and the mostly subtidal *Arbacia lixula* – were submitted to a protocol of stepwise (rate of 2–3 psu/h) dilution, down to 15 psu, or concentration, up to 45 psu, of control seawater (35 psu). Coelomic fluid samples were obtained every hour. The seawater dilution experiment lasted 8 h, while the seawater concentration experiment lasted 6 h. Significant gradients (40–90% above value in 15 psu seawater) for osmolality, sodium, magnesium, and potassium were shown by *L. variegatus* and *E. lucunter*. *A. lixula* showed the smallest gradients, displaying the strongest conforming behavior. The esophagus of the three species was challenged *in vitro* with 20 and 50% osmotic shocks (hypo- and hyperosmotic). *A. lixula*, the most “conforming” species, showed the highest capacity to avoid swelling of its tissues upon the –50% hyposmotic shock, and was also the species less affected by salinity changes concerning the observation of spines and ambulacral feet movement in the whole-animal experiments. Thus, the most conforming species (*A. lixula*) displayed the highest capacity to regulate tissue water/volume, and was also the most euryhaline among the three studied species. In addition, tissues from all three species swelled much more than they shrank under osmotic shocks of same magnitude. This distinct trend to gain water, despite the capacity to hold some gradients upon seawater dilution, helps to explain why echinoderms cannot be fully estuarine, or ever enter fresh water.

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1. Introduction

Echinoderms are exclusively marine; they have spread and diverged down to abyssal depths, but when close to continents, remained subtidal in shallow depths or in the interface to terrestrial habitats, frequently occupying intertidal rocky coasts. However, they are generally absent from estuaries, the boundaries between seawater and fresh water. Interestingly, ophiuroids are the most frequent echinoderms in estuarine brackish waters (Binyon, 1966). Definitely, echinoderms are never found in fresh water (Fell, 1966; Turner and Meyer, 1980). This pattern has been long recognized, and is associated to an osmoconforming strategy, allied to relative stenohalinity (e.g., Sabourin and Stickle, 1981; Diehl, 1986; Stickle and Diehl, 1987; Vidolin et al., 2007). The stenohalinity derives from high permeabilities of the body wall and ambulacral system feet, and other epithelia, as well as from the lack of an excretory system able to perform active vectorial salt transport (e.g., Hyman, 1955; Binyon, 1966; Cavey and Märkel, 1994; Warnau et al., 1998; Santos-Gouvea and Freire, 2007).

High water and ion permeability and a fresh water existence in fact do not fit together. Animals can be highly permeable in estuaries, if they have some escape strategies to employ during low tides (e.g., barnacles or bivalve mollusks), or if they have a remarkable cell volume regulatory capacity, as for instance seen in polychaetes (e.g., Kirschner, 1991; Willmer et al., 2005). Echinoderms seem to lack both capabilities. Thus, they exhibit low tolerance to salinity reduction, i.e., mortality, (e.g., Lawrence, 1975; Junqueira et al., 1997; Freire et al., 2011; Meng et al., 2011), or else disturbance of several functions. For example, low salinity disturbs larval survival and proper development (*Echinometra lucunter* in Metaxas, 1998), reproduction (genera *Echinometra* and *Diadema* in Lessios, 1981), the immune response (*Asterias rubens* in Coteur et al., 2004), regeneration (*Ophiophragmus filograneus*, in Talbot and Lawrence, 2002), locomotion or the righting response (*Luidia clathrata* in Ellington and Lawrence, 1974, and *Patiriella mortenseni* in Barker and Russell, 2008).

However, despite being considered stenohaline osmoconformers, echinoderms frequently withstand wide variations in salinity either in their environments (especially intertidal), or experimentally in the laboratory (e.g., Binyon, 1966; Stickle and Ahokas, 1974; Stickle and Denoux, 1976; Shumway, 1977; Diehl and Lawrence, 1984; Barker and Russell, 2008; Freire et al., 2011). When tolerating salinity variations, they show some transient gradients between their coelomic

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fluid and external water due to a certain buffering of the extracellular concentrations, or they simply survive the wide extracellular changes. Moreover, ample evidence of species differences – both in tolerance and in the establishment of extracellular gradients – is available from the literature (Stickle and Ahokas, 1974; Diehl, 1986; Stickle and Diehl, 1987; Vidolin et al., 2007; Barker and Russell, 2008; Freire et al., 2011). In addition, reports of cell volume or tissue water regulation in osmotically challenged echinoderms are scarce and conflicting (e.g., Madrid et al., 1976; Diehl and Lawrence, 1985; Diehl, 1986; Foglietta and Herrera, 1996).

In this study, the responses of the echinoids *Lytechinus variegatus* (can occur next to estuarine areas), *E. lucunter* (rocky intertidal), and *Arbacia lixula* (mostly subtidal) to osmotic challenges were comparatively investigated. The hypotheses that drove this study were: 1) *L. variegatus* and *E. lucunter*, being larger inhabitants of more unstable environments, will display larger extracellular gradients between the coelomic fluid and external seawater than *A. lixula*, the smaller subtidal species; 2) *L. variegatus* will display higher gradients than *E. lucunter*, according to previous data from our laboratory (Vidolin et al., 2007; Freire et al., 2011). However, being more “conforming” than the other two species (Vidolin et al., 2007), *A. lixula* will possibly have a larger capacity for cell volume maintenance (here evaluated as tissue hydration, Foster et al., 2010).

2. Materials and methods

2.1. Species habitats and sampling sites

The species chosen for this study were 1) the green sea urchin *L. variegatus*, which is a soft substrate shallow subtidal species, but reported to occur near estuaries (Drifmeyer, 1981; Ernest and Blake, 1981; Junqueira et al., 1997); 2) the rock-boring urchin *E. lucunter*, a typically intertidal species on rocky coasts which is also found down to depth of 45 m (Grinbaum et al., 1978; Castro et al., 1995; Hendler et al., 1995; Sánchez-Jérez et al., 2001); and 3) the black sea urchin *A. lixula*, an essentially subtidal urchin, occurring down to a depth of 50 m (Chelazzi et al., 1997; Benedetti-Cecchi et al., 1998), usually reported not to occur exposed during low tides (Castro et al., 1995; Hendler et al., 1995; Bulleri et al., 1999).

L. variegatus (Lamarck, 1816) (total n = 66; largest test diameter of 111.4 ± 2.6 mm, 10 measured individuals) were collected through snorkelling or scuba diving in the city of Bombinhas, Santa Catarina State, Brazil (27° 08' 28–48" S, 48° 28' 42–54" W), never from intertidal areas, only submerged, distant from intertidal coasts. *E. lucunter* (Linnaeus, 1758) (total n = 72, largest test diameter 139.2 ± 4.7 mm, 12 measured individuals), and *A. lixula* (Linnaeus, 1758) (total n = 69, largest test diameter 84.4 ± 1.9 mm, 10 measured individuals) were collected during low tide from rocky coasts, also in Bombinhas, or else from the city of Penha (26° 46' 27" S, 48° 36' 02" W), also in Santa Catarina State, Brazil, from 2006 to 2012. On the rocky coasts of the studied sites, *E. lucunter* was abundantly observed in intertidal areas, exposed to the air during low tides, sometimes in rock crevices (Santos-Gouvea and Freire, 2007; Freire et al., 2011). *A. lixula* was collected along the rocky coasts, but always in subtidal, non-exposed areas. On only one occasion four individuals were observed exposed to the air (Castellano, pers. obs.).

2.2. Transport and acclimation to laboratory conditions

All urchins were transported in styrofoam boxes, wrapped by green kelp to retain moisture, for up to 3 h, to the laboratory in Curitiba, State of Paraná. In the laboratory they were acclimated for approximately 5–7 days in a stock tank (160 L) containing seawater (salinity 33–35 psu at 22 ± 2 °C, pH 7.5–8.0), under biological filtration and constant aeration, and natural photoperiod (12 h light:12 h dark). *L. variegatus* and *E. lucunter* were fed every two days with large

fragments of *Ulva* sp., while *A. lixula* specimens were fed with small pieces of fish meat. All procedures described with the urchins were approved by the Committee of Ethics on Animal Experimentation of the Federal University of Paraná: certificates number 254 and 359, issued respectively on August 28, 2007 and April 7, 2009.

2.3. Experiments of stepwise dilution or concentration of seawater

Each urchin used for the seawater (SW) dilution experiment was individually placed inside a 1.5 L aquarium. It was initially exposed to control full-strength SW of 35 psu. After 1 h, the water was replaced with slightly diluted SW of 33 psu. Every hour, water was removed and replaced by further diluted SW: 30, 27, 24, 21, 18, and finally 15 psu, for 8 h. The same procedure was adopted using other specimens, for the SW concentration experiment: initial control in salinity 3 psu, then 37, 39, 41, 43, and finally 45 psu, taking a total time of 6 h for the whole SW concentration experiment. Ten sea urchins in total were used for the SW dilution and 10 for the SW concentration experiment, for each species. Dilution of SW was achieved through the addition of appropriate volumes of filtered tap water (activated charcoal and cellulose filters; Aqualar, Brazil); salinity was raised in concentrated SW through the addition of commercially available marine salt to full-strength SW. Salinities were always verified using a refractometer (Shibuya S28, Japan). At the end of every hour, before changing the water, a sample of perivisceral coelomic fluid (~500 µL) was withdrawn through puncture of the peristomial membrane using an hypodermic insulin syringe. A sample of the aquarium water was also taken at this moment. Both coelomic fluid and water samples were frozen at -20 °C and maintained frozen until assayed for osmolality and ions.

2.4. Behavioral observations

The “Righting-Time Response” test was employed in order to verify the wellbeing of the urchins during the progressive dilution or concentration of SW. The test was always performed 30 min after changing the experimental water. The urchin was then manually turned “upside-down”, with its aboral side facing the substrate. The total time taken by the urchin to return with its oral side to the substrate (bottom of the glass aquarium) was recorded. Normal behavior was considered when animals righted themselves within 30 min. At this time the stiffness and degree of movement of the spines and ambulacral feet were also recorded, as well as the response of the urchin to a stimulus: gently touching the urchin with tweezers, and observing the movement of spines and ambulacral feet in the direction of the tweezers. A qualitative arbitrary scale of intensity of the response was set in order to detect the effects of changing salinities on the behaviour of the urchins. Responses are indicated in an arbitrary scale, ranging from the absence of a behaviour or response (–) to its highest intensity, typically as manifested in control full-strength seawater (+++); ± and + are intermediary, subjective levels of the response. All those observations were indicative of the general wellbeing of the urchins.

2.5. Assays of osmolality and ions in the coelomic fluid and water of the aquaria

Osmolality was assayed in undiluted coelomic fluid or water samples using a vapor pressure micro-osmometer (VAPRO Wescor 5520). Chloride and magnesium ions were assayed in duplicates in samples diluted in deionized water, using Labtest colorimetric kits, and absorbance read respectively at 470 and 505 nm (Ultraspec 2100 PRO Amersham Pharmacia Biotech, Sweden). Ions sodium and potassium were assayed through flame photometry (B462 Micronal, Brazil), also in samples diluted in deionized water. Osmolality and ions measured in the aquaria water samples are provided in Table 1, where they have been compared to expected values calculated from standard SW values in Prosser (1973). The general agreement between calculated

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