Osmotic stability of the coelomic fluids of a sea-cucumber (*Holothuria grisea*) and a starfish (*Asterina stellifera*) (Echinodermata) exposed to the air during low tide: a field study

Estabilidade osmótica dos fluídos celômicos de um pepino do mar (*Holothuria grisea*) e de uma estrela-do-mar (*Asterina stellifera*) (Echinodermata) expostos ao ar durante a maré baixa: um estudo de campo

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Echinoderms are exclusively marine invertebrates, being largely considered as stenohaline and osmoconformers, thus unable to tolerate wide variations in sea water salinity or to withhold significant osmotic and ionic gradients with respect to sea water. However, these animals are found in the intertidal zone, inside tide pools, where salinity can increase due to evaporation from exposure to intense sunshine, or decrease from heavy rainfall or fresh water runoffs. They can even get totally exposed to the air, subject to significant water loss (Stickle & Ahokas 1974; Stickle & Denoux,

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1976). These are observations which have been already pointed out by several previous studies, with resulting questioning of their widely claimed stenohalinity or lack of ionic regulation (Stickle & Ahokas 1974; Stickle & Diehl 1987; Bishop *et al.*, 1994). The conclusion that can be drawn is that echinoderms, at least those inhabiting intertidal or estuarine areas, must either have some osmoregulatory mechanism, even slight, or have lower body wall permeabilities than generally assumed for marine osmo-ionic conformer animals, or else, have tissues with cells of considerable volume regulatory capacity. The strong capacity to regulate cell volume when facing salinity/ osmotic stress seems probable, as several studies have demonstrated the lack of significant osmotic gradients between the coelomic fluid of echinoderms and ambient sea water (Stickle & Ahokas, 1974; Foglietta & Herrera, 1996). Few works have been conducted *in situ*, as pointed out by Stickle and Denoux (1976). And to our knowledge, this gap has not been filled in the last 27 years. In that study, conducted in Alaska, the authors have focused on several intertidal invertebrates (Stickle & Denoux, 1976), and found no indication of body-fluid osmoconcentration when the specimens were exposed to the air at low tide. Thus, the question asked by the present study was: What happens to coelomic fluid osmolality when tropical intertidal echinoderms are faced with air exposure during low tide in their natural habitat?

MATERIALS AND METHODS

This study was conducted during low tide in a rocky shore (Quilombo beach, 26° 46‘S, 48° 38‘W, Penha, Santa Catarina State, Brazil. Coelomic fluid samples (200-500 µl) have been withdrawn through body wall puncture, using a disposable insulin syringe, from the starfish *Asterina stellifera* (ambulacral area) and the sea cucumber *Holothuria grisea* either exposed to the air during low tide, or submerged in surrounding sea water. At the same time points, one water sample from the surrounding sea water (salinity of 31-33 ‰) was also collected. The same animals were sampled in a same day of data collection, without control over identification of the

specimens. Due to their low mobility, it could be ascertained that the same group of animals were sampled over the time course of each individual date. In the first day of data collection, conducted in September 2001, it was at first cloudy, then it started to rain on the exposed animals (air temperature ~17° C). In the second day of data collection, conducted in March 2002, the weather was clear and there was intense sunshine (air temperature ~30° C). Animals were sampled at the start of their exposure to the above conditions, and again after 1 hour of exposure to the same conditions. Coelomic fluid and water samples were stored in ice in the field and were immediately brought to the laboratory where they were frozen to -20 °C until assayed for osmolality. Osmolality was determined using a vapor pressure micro-osmometer VAPRO Wescor 5520 (USA). Data are presented in the Figure as mean ± standard error of the mean. The number of exposed animals sampled at each weather condition was 6 for *H. grisea* and *A. stellifera*. The number of submerged animals sampled was 29 for *H. grisea* and 24 for *A. stellifera*. Data were analysed through unpaired Student´s *t* using the statistical software Sigma-Stat® version 1.03, Jandel Corporation, always with significance set at 0.01.

RESULTS

Coelomic fluid osmolality of holothurians submerged in sea water was somewhat higher than the osmolality of sea water (Fig. 1A). Coelomic fluid osmolality of holothurians exposed to the air even after 1 hour under cloudy or rainy conditions were lower than that of animals submerged in sea water. In contrast, after 1 hour of air exposure under sunshine, coelomic fluid osmolality increased (Fig. 1A).

Coelomic fluid osmolality of starfishes submerged in sea water were also higher than sea water osmolality (Fig. 1B). As it happened with the holothurians, again starfish exposed to the air under cloudy or rainy conditions displayed lower osmolalities than controls submerged in sea water (Fig. 1B). Differences between the osmolalities displayed by the coelomic fluid samples of both

echinoderms appeared only after 1 hour of exposure to the rain (Fig. 1). Independent of statistical differences, there was a trend repeated for both species, of a slight reduction in osmolality when animals were exposed to cloudy or rainy conditions, and of no change in osmolality when animals were exposed to sunshine.

DISCUSSION AND CONCLUSIONS

Osmolality of the coelomid fluids of asteroids and holothuroids studied here were remarkably stable (despite statistically significant differences indicated) in individuals exposed to the air, as also found in a similar *in situ* study, conducted in Alaska (STICKLE & DENOUX, 1976), for several other echinoderms: the holothurians *Eupentacta quinquesemita* and *Cucumaria vegae*, the starfish *Evasterias troschelii*, and the sea-urchin *Strongylocentrotus droebachiensis*. These authors discuss air exposure and air temperature, but do not mention any rain or sunshine during air exposure (STICKLE & DENOUX, 1976). The temperature recorded here in the March data collection date (~30º C), additionally with direct sunhine, would be expected to represent a stronger osmotic challenge for the echinoderms when compared to the air exposure at 25º C in the study conducted in Alaska (STICKLE & DENOUX, 1976). In contrast to these data on echinoderms, in field experiments (air exposure during low tide in warm climate) using intertidal limpets, significant water loss (3-19%) has been observed (DAVIES, 1969), or either significant osmoconcentration of body fluids (SEGAL & DEHNEL, 1962). This is a surprising result, considering that these animals have protective shells.

As would be expected, a reduction in osmolality, however small, was observed when it rained on the exposed echinoderms. Nevertheless, the same happened when the weather was cloudy, and the air close to the animals extremely saturated with moisture. This last result is difficult to explain, and would require additional experiments for clarification. The slight increase already after the first minutes of exposure to sunshine would also be expected. In none of the cases was there an effect from 1 hour of air exposure,

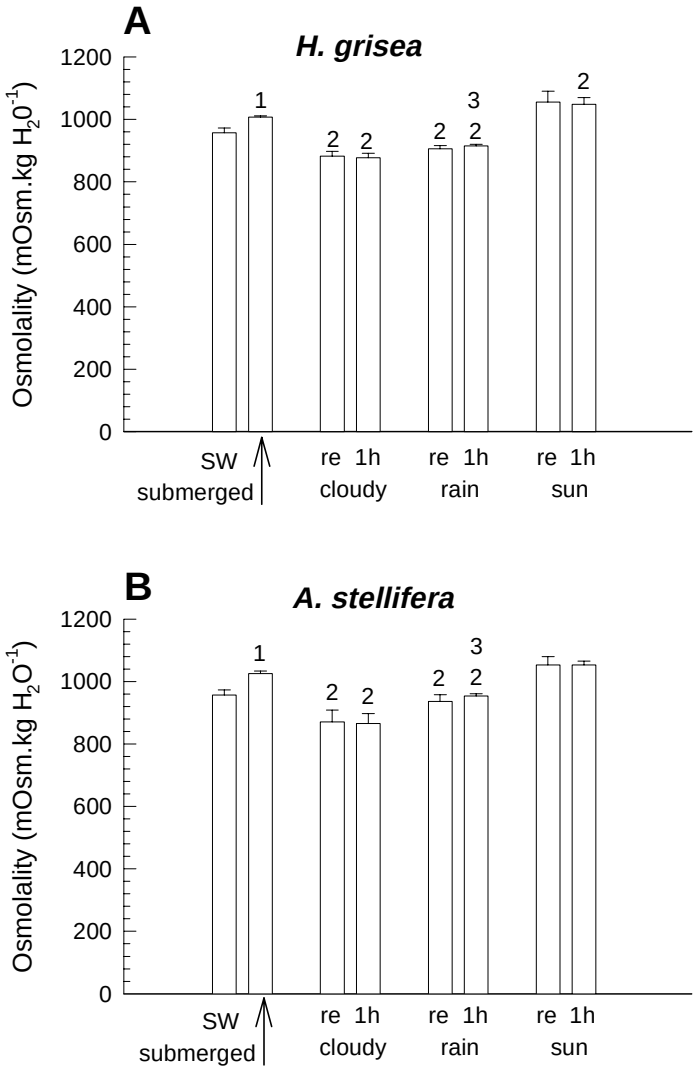

Fig. 1. Osmolality (mean ± standard error of the mean, mOsm.kg H_2O^{-1}) of the coelomic fluid of *H. grisea* (**A**) and *A. stellifera* (**B**) in animals submerged in sea water (arrow), exposed to the air under cloudy weather, exposed to the air under rain, or exposed to the air under sunshine; re = recently exposed, 1h = after 1 hour of exposure. The leftmost bar on each graph represents osmolality of surrounding sea water (SW). Significant differences: ($p<0.01$): 1 = sea water x coelomic fluid of animals submerged in sea water; 2 = coelomic fluid of animals submerged in sea water x coelomic fluid of exposed animals, 3 = coelomic fluid of *H. grisea* x coelomic fluid of *A. stellifera*, for the same experimental condition.

suggesting that as the animal detects it is exposed to the air, it can somehow immediately decrease its body wall permeability. In summary, despite the fact that the expected trends have been observed (except for the cloudy conditions), the differences were very slight, strongly suggesting a mechanism of detection of air exposure, with resulting reduction in body wall permeability. This conclusion of low body wall permeabilities, with the additional possibility of a certain degree of regulation of that apparent permeability by the animal supply material to the questioning of the well known concept of echinoderms as stenohaline osmoconformers. Physiological field studies are rare for intertidal echinoderms, but can contribute to the unravelling of details of their successful adaptation to one of the physiologically most challenging marine habitats.

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RESUMO

Animais de entre-marés pode ser expostos ao ar durante a maré baixa, por pelo menos 1-2 horas. Os animais expostos ao ar são susceptíveis a perda de sal e/ou entrada de água durante chuva intensa, ou perda de água pela ação de dessecação do sol. A osmolalidade de amostras de fluido celômico obtidas do pepino-do-mar *Holothuria grisea* e da estrela-do-mar *Asterina stellifera* expostas ao ar, ou de animais controles imersos na água do mar adjacente foi determinada. As amostras foram obtidas imediatamente após a exposição ao ar, e novamente após uma hora de exposição ao ar, durante a maré baixa no campo, em tempo nublado, chuvoso, ou ensolarado, na Praia rochosa do Quilombo, Penha, Sul do Brasil. Uma hora de exposição a qualquer das condições climáticas indicadas não alterou a osmolalidade dos fluidos celômicos. Houve pequena redução nas osmolalidades dos fluidos celômicos durante a exposição ao ar com precipitação de chuva. Sugere-se que estes equinodermas possam imediatamente detectar sua exposição ao ar, e possam então reduzir a permeabilidade osmótica de sua parede do corpo, para evitar perda de água para o ar ou entrada de água/saída de sal durante a chuva.

PALAVRAS CHAVE: *Asterina*, equinodermas, estudo-de-campo, fluido celômico, *Holothuria*, osmorregulação

SUMMARY

Intertidal animals can be exposed to the air during low tide, for at least 1-2 hours. Animals exposed to the air are subject to salt loss (or water gain) from heavy rains or volume loss from the desiccating action of the sun. Coelomic fluid samples obtained from the sea-cucumber *Holothuria grisea* and the starfish *Asterina stellifera* exposed to the air or from control animals submerged in surrounding sea water have been assayed for osmolality. Samples were obtained right after air exposure and again after 1 hour of exposure to the air during low tide in the field, either under cloudy, rainy or sunny weather conditions, in the rocky beach of Quilombo, Penha, Southern Brazil. One hour of exposure to any of the conditions did not change coelomic fluid osmolalities. There was a slight reduction in coelomic fluid osmolalities upon air exposure during rainfall. It is suggested that these echinoderms can somehow immediately detect air exposure and reduce their body wall permeability to avoid water loss or water influx/salt loss during rainfall.

KEY WORDS: *Asterina*, coelomic fluid, echinoderm, field study, *Holothuria*, osmoregulation

RÉSUMÉ

Animaux d'entre-marées peuvent êtres exposés a l'air libre pendant le reflux de la marée, pour environ une ou deux heures seulement. Ces animaux, quand exposés a l'air libre, sont susceptibles de perdre du sel et d'absorber de l'eau pendant une période de pluie intense. Par contre, ils peuvent perdre de l'eau si soumis a l'action de dessèchement due a une éxposition au soleil. On a réussi a determiner l'osmolalité d'échantillons du fluide celomique obtenus du Pépin-de-mer *Holothuria grisea* et de l'Étoile-de-mer *Asterina stellifera* exposés a l'air libre, e d'animaux-controles immergés dans l'eau de mer voisin. Les échantillons ont été obtenus tout de suite après l'exposition à l'air et, une seconde fois, après une heure d'exposition à l'air libre, pendant la durée de la marée basse, soit sous la pluie, soit au soleil ou soit sous un ciel ombrageux, à la plage rocailleuse de Quilombo, Penha, au sud du Brésil. Une heure

d'éxposition à n'importe quelles conditions climatiques indiquées, n'ont pas pu altérer l'osmolalité des fluides celomiques, ce que sugère la conclusion que ces échinodermes peuvent détecter immédiatement sa exposition à l'air libre et peuvent tout de suite réduire la permeabilité osmotique de la membrane que recouvre son corps pour éviter perdre d'eau et, de la même façon, reduire l'absortion de l'eau pendant la pluie. On a observé une petite réduction de fluides celomiques pendant l'exposition a l'air, avec ocurrence de pluie.

MOTS CLÉS: *Asterina*, échinoderme, fluide celomique, *Holothuria*, osmoregulation

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