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Buffer capacity of the coelomic fluid in echinoderms

Marie Collard [a,b,*,1], Kim Laitat [a,1], Laure Moulin [a,c], Ana I. Catarino [a], Philippe Grosjean [c], Philippe Dubois [a]

[a] Laboratoire de Biologie Marine, Université Libre de Bruxelles, 50 avenue F.D. Roosevelt, B-1050 Brussels, Belgium
[b] Laboratorium voor Analytische Chemie, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussels, Belgium
[c] Laboratoire d'Ecologie Numérique des Milieux Aquatiques, Université de Mons-Hainaut, 6 Avenue du Champ de Mars, B-7000 Mons, Belgium

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ABSTRACT

The increase in atmospheric CO_2 due to anthropogenic activity results in an acidification of the surface waters of the oceans. The impact of these chemical changes depends on the considered organisms. In particular, it depends on the ability of the organism to control the pH of its inner fluids. Among echinoderms, this ability seems to differ significantly according to species or taxa. In the present paper, we investigated the buffer capacity of the coelomic fluid in different echinoderm taxa as well as factors modifying this capacity. Euechinoidea (sea urchins except Cidaroidea) present a very high buffer capacity of the coelomic fluid (from 0.8 to 1.8 mmol kg^{-1} SW above that of seawater), while Cidaroidea (other sea urchins), starfish and holothurians have a significantly lower one (from −0.1 to 0.4 mmol kg^{-1} SW compared to seawater). We hypothesize that this is linked to the more efficient gas exchange structures present in the three last taxa, whereas Euechinoidea evolved specific buffer systems to compensate lower gas exchange abilities. The constituents of the buffer capacity and the factors influencing it were investigated in the sea urchin *Paracentrotus lividus* and the starfish *Asterias rubens*. Buffer capacity is primarily due to the bicarbonate buffer system of seawater (representing about 63% for sea urchins and 92% for starfish). It is also partly due to coelomocytes present in the coelomic fluid (around 8% for both) and, in *P. lividus* only, a compound of an apparent size larger than 3 kDa is involved (about 15%). Feeding increased the buffer capacity in *P. lividus* (to a difference with seawater of about 2.3 mmol kg^{-1} SW compared to unfed ones who showed a difference of about 0.5 mmol kg^{-1} SW) but not in *A. rubens* (difference with seawater of about 0.2 for both conditions). In *P. lividus*, decreased seawater pH induced an increase of the buffer capacity of individuals maintained at pH 7.7 to about twice that of the control individuals and, for those at pH 7.4, about three times. This allowed a partial compensation of the coelomic fluid pH for individuals maintained at pH 7.7 but not for those at pH 7.4.

1. Introduction

Anthropogenic emissions of carbon dioxide (CO_2) are inducing an important increase in the atmospheric CO_2 concentration which in turn modifies seawater chemistry of the oceans, lowering its pH and shifting the carbonate equilibrium (IPCC, 2007; Orr, 2011). These two effects are commonly regrouped under the name of ocean acidification. The pH decrease is predicted to reach 0.3–0.4 U by 2100 and 0.8 by 2300, following the IPCC "business-as-usual" IS92a scenario (Caldeira and Wickett, 2003, 2005; IPCC, 2007).

The increased CO_2 concentration in seawater impacts marine organisms directly as CO_2 enters the organisms by diffusion inducing hypercapnia (i.e. CO_2 accumulation in the internal fluids) and indirectly through acidosis (internal pH decrease). Hypercapnia and/or acidosis may affect different physiological processes such as calcification, nutrition and metabolism (Pörtner, 2008; Melzner et al., 2009). In order to avoid this, organisms need to maintain the homeostasis of their inner fluids through different processes such as respiration, circulation, ionic and acid–base regulation. The maintenance of intracellular pH is crucial to numerous enzymatic reactions (Pörtner, 2008). For that purpose, intracellular protons are transported into the extracellular fluids. Therefore, the buffer capacity of the latter is considered essential for the maintenance of intracellular pH. This buffer capacity depends on both the capability of substances present in the fluid to transfer free protons into a non-dissociated state and the available volume of extracellular fluid (Heisler, 1989). If extracellular pH decreases, additional energy will be required to transport, first, the protons from intra- to extracellular compartments and, second, from the extracellular compartment to the external medium. Consequently, less energy can be allocated to other processes such as growth or reproduction (Pörtner et al., 1998; Pörtner et al., 2000; Melzner et al., 2009).

* Corresponding author at: Laboratoire de Biologie Marine, Université Libre de Bruxelles, 50 avenue F.D. Roosevelt, B-1050 Brussels, Belgium. Tel.: +32 2 650 37 86; fax: +32 2 650 27 96.
E-mail address: marie.collard9@gmail.com (M. Collard).
[1] These authors contributed equally to this work.

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Metazoans have two buffer systems to compensate for fluctuations of the extracellular pH: a bicarbonate buffer system and a non-bicarbonate buffer system (Heisler, 1986). The former is the most commonly used as it is a low cost and fast system that may be used in order to buffer a too large concentration of protons. However, this system is more or less sustainable, depending on the species, as an increase in the proton concentration in the extracellular fluid will result in a higher concentration in CO_2 which will have to be eliminated later. The efficiency of this system will depend on the physiological bicarbonate accumulation potential of the species and its capacity to evacuate CO_2 (Heisler, 1986; Melzner et al., 2009). The second system, the non-bicarbonate buffer system, can also allow the regulation of the acid–base balance of the extracellular fluids by capturing the protons liberated by the hydration of CO_2. From non-bicarbonate substances, only those whose p*K* is close to that of the physiological pH of the organisms may play a role. This principally includes residues of polypeptide chains, e.g. histidine, cysteine and terminal NH_2 groups and phosphates (either inorganic secondary phosphate or miscellaneous organic phosphate compounds). Differences in the p*K* values associated to these different compounds allow a large range of pH to be buffered. However, this system includes the production of proteins and other compounds, which requires a high amount of energy (Heisler, 1986; Melzner et al., 2009). By combining both of these systems, some organisms are capable of partially or totally compensate the extracellular acidosis induced by seawater acidification. For instance, Gutowska et al. (2010) showed that the cuttlefish *Sepia officinalis* partially compensates its extracellular pH (pH_e) by accumulating bicarbonate ions in its internal fluid. This was also observed in the mollusk *Patella vulgata* where a passive dissolution of the shell contributes to a compensation of pH_e (Marchant et al., 2010).

Many echinoderms live in frequently changing environments such as intertidal and upwelling zones. For these organisms, variations in pH and temperature are a regular or frequent feature (Truchot and Duhamel-Jouve, 1980; Feely et al., 2008; Moulin et al., 2011). Therefore, it might be expected that these organisms have the capacity to bear these fluctuating parameters. Moreover, numerous echinoderm species occur under the saturation horizon for magnesium calcite (David et al., 2005; Hall-Spencer et al., 2008; Sewell and Hofmann, 2011), indicating a possible tolerance to corrosive seawaters. Finally, recent studies have delivered results suggesting that adult echinoderms may be able to cope with seawater pH decrease within the scope of ocean acidification (see Dupont et al., 2010 for review; Catarino et al., 2012; Dupont and Thorndyke, 2012; Stumpp et al., 2012). Spicer et al. (1988) showed that, during air exposure, the sea urchins *Echinus esculentus* and *Psammechinus miliaris* are able to compensate acidosis for a short period (several hours) as no extracellular pH decrease was observed. However, Miles et al. (2007) observed that *P. miliaris* was unable to regulate its acid–base balance if submitted to a low pH (7.4, 6.63 and 6.16) for about a week. These authors hypothesized that acidosis of the coelomic fluid induced a dissolution of the skeleton (source of bicarbonate ions), which resulted in a slight compensation of the coelomic fluid pH (pH_{CF}). However, other authors did not observe any evidence of skeleton dissolution in another sea urchin species (Burnett et al., 2002). On the contrary, two studies reported that the sea urchin, *Strongylocentrotus droebachiensis*, maintained at reduced seawater pH during several weeks, is able to fully or partly compensate the acidosis (Dupont and Thorndyke, 2012; Stumpp et al., 2012). Over similar time scales, acidosis of the coelomic fluid in the starfish *Asterias rubens* and *Leptasterias polaris* remained uncompensated (Appelhans et al., 2012; Dupont and Thorndyke, 2012).

The main circulatory medium of echinoderms is the coelomic fluid (CF), i.e. the fluid enclosed in the main body cavity, that, together with the water vascular system, ensures gas transportation (Farmanfarmaian, 1966). Although echinoderms are osmoconformers, their CF ionic composition slightly differs of that of seawater (Bialaszewicz, 1933; Binyon, 1972). Their pH_{CF} is usually 0.5 to 1.5 U lower than the pH of surrounding seawater because of a high pCO_2 of metabolic origin (Cole, 1940; Hyman, 1955; Farmanfarmaian, 1966). Echinoderms also have organic compounds present in their CF , mainly amino acids, reduced sugars, proteins, lipids and nitrogenous wastes. This composition is directly dependent on the nutritional state of the organism as the CF serves as a vessel for nutrient transport (Ferguson, 1964; Holland et al., 1967; Binyon, 1972). Finally, the CF of echinoderms contains circulating cells, i.e. coelomocytes, whose functions range from metabolite transport to immunity (Endean, 1966). Even though it was shown previously that the CF of echinoderms may exhibit a much higher buffer capacity than seawater (sea urchins CF exhibits the highest buffer capacity followed by sea stars and sea cucumbers) (Collip, 1920; Gellhorn, 1926; Sarch, 1931; Koller and Meyer, 1933; Meyer, 1935), nothing is known about the environmental and physiological factors influencing this property. The origin of the buffer capacity is unknown, and so is its nature. This increased buffer capacity could explain, for a part, the occurrence of echinoderms in low pH environments.

The goal of the present study was to investigate the factors influencing the CF buffer capacity of echinoderms and to get a first insight into the source of this capacity. For that purpose, we have scanned a range of species to assess this capacity in the phylum. We also studied the effects of nutrition on the CF buffer capacity of the sea urchin *Paracentrotus lividus* and the starfish *A. rubens* and how it is distributed among the CF constituents. The effect of reduced seawater pH on this property was investigated in *P. lividus*.

2. Materials and methods

2.1. pH, alkalinity and buffer capacity measurements

In order to measure pH and alkalinity (A_T), coelomic fluid (CF) was sampled from each individual using a syringe and was transferred to an Eppendorf tube. The pH and the electromotive force (emf) were measured immediately after sampling at the same temperature as the seawater hosting the animals using a Metrohm pH meter fitted with a microelectrode (826 pH mobile, microelectrode reference 6.0224.100; Metrohm, Switzerland). The electrode had been previously calibrated with CertiPUR® Buffer solutions pH 4.00 and 7.00 (Merck, Darmstadt, Germany). Taking into account the similar composition of CF and that of seawater (SW) (Stickle and Diehl, 1987), the pH measurements were converted in total scale according to DelValls and Dickson (1998) calibration method using standard buffers of known pH, 2-aminopyridine/HCL (AMP) and tris/HCL (TRIS) (kindly provided by the Department of Astrophysics, Geophysics and Oceanography of the University of Liège, Belgium). The alkalinity of SW ($A_{T\text{-}SW}$) and the alkalinity of the CF ($A_{T\text{-}CF}$) were measured using a micromeasurement technique based on a potentiometric titration (Gran, 1952). The titration took place by first adding 5 μL of HCl 0.1 mol L^{-1} (Merck) and then 1 μL at a time to an initial sample of 0.5 mL until reaching a pH lower than 3.00.

In order to verify that the measured increase in $A_{T\text{-}CF}$ is efficient in the physiological range of pH, the CF of four *P. lividus* (3rd batch, for origin see Table 1) was titrated by adding 0.5 μL of HCl 0.1 mol L^{-1} at a time.

The method was tested by using total alkalinity standard certified material provided by Andrew G. Dickson's Oceanic Carbon Dioxide Quality Control Laboratory (batch number 94). The reproducibility of the method was also tested on the CF of *P. lividus* (first batch, Table 1): $A_{T\text{-}CF}$ was measured 3 times for three independent individuals.

In order to make the results comparable between individuals of different species and/or from different origins, all measures were converted into delta alkalinity (ΔA_T) which was calculated as follows:

$$\Delta A_T = A_{T\text{-}CF} - A_{T\text{-}SW}$$