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RESEARCH ARTICLE

Acid–base balance and metabolic response of the sea urchin *Paracentrotus lividus* to different seawater pH and temperatures

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Abstract
Purpose In order to better understand if the metabolic responses of echinoids could be related to their acid–base status in an ocean acidification context, we studied the response of an intertidal sea urchin species, *Paracentrotus lividus*, submitted to low pH at two different temperatures.
Methods Individuals were submitted to control (8.0) and low pH (7.7 and 7.4) at 10°C and 16°C (19 days). The relation between the coelomic fluid acid–base status, the RNA/DNA ratio of gonads and the individual oxygen uptake were studied.
Results The coelomic fluid pH decreased with the aquarium seawater, independently of temperature, but this explained only 13% of the pH variation. The coelomic fluid showed though a partial buffer capacity that was not related to skeleton dissolution ($[Mg^{2+}]$ and $[Ca^{2+}]$ did not differ between pH treatments). There was an interaction between temperature and pH on the oxygen uptake (V_{O2}) which was increased at pH 7.7 and 7.4 at 10°C in comparison with controls, but not at 16°C, indicating an upregulation of the metabolism at low temperature and pH. However, gonad RNA/DNA ratios did not differ according to pH and temperature treatments, indicating that even if maintenance of physiological activities has an elevated metabolic cost when individuals are exposed to stress, they are not directly affected during short-term exposure. Long-term studies are needed in order to verify if gonad production/growth will be affected by low pH seawaters exposure.

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1 Introduction

Since the industrial revolution, ocean surface pH has been reduced by approximately 0.1 U due to seawater uptake of anthropogenic CO_2 emissions, a phenomenon known as ocean acidification. In the near future, pH is expected to decrease 0.3–0.5 U by 2100 and 0.7–0.8 by 2300 (Caldeira and Wickett 2003, 2005; IPCC 2007). The metabolism of marine organisms can be depressed when organisms are exposed to high CO_2 conditions (environmental hypercapnia) (Pörtner 2008; Melzner et al. 2009), and processes such as reproduction, calcification, and growth are vulnerable to acidification (Doney et al. 2009; Fabry et al. 2008; Pörtner 2008; Melzner et al. 2009; Hofmann et al. 2010; Hofmann and Todgham 2010). The development of early stages of some marine organisms have revealed vulnerabilities when raised in lower pH seawaters such as decreased cleavage rates, a reduction of the larva size and/or a delay in their development (Kurihara 2008; Dupont et al. 2010). In what concerns adults, ocean acidification effects are generally negative, but it can be hard to establish patterns in responses and sensitivities (Kroeker et al. 2010). Furthermore, there seems to be an increasing body of evidence that even closely related species and/or different life stages respond differently to seawater pH levels within ocean acidification near future predictions (Melzner et al. 2009; Ries et al. 2009; Dupont et al. 2010).

The maintenance of extracellular pH is considered to be crucial in protecting individuals against hypercapnia-induced disturbances (Heisler 1989; Seibel and Walsh

2003; Pörtner 2008). One of the main acid–base regulation mechanisms is associated with active ion transport achieved across specialized epithelia such as gills, renal or digestive tissues, and therefore also with osmoregulation (Whiteley et al. 2001; Seibel and Walsh 2003; Pane and Barry 2007; Melzner et al. 2009). Hypometabolic and osmoconformer organisms should be less able to cope with ocean acidification effects, precisely because they lack ion-regulatory machinery that could protect physiological fluids against hypercapnia (Pane and Barry 2007; Pörtner 2008; Melzner et al. 2009), possibly explaining the elevated sensitivity of some marine invertebrates to ocean acidification, with a decrease of extracellular pH linked to metabolic depression (Reipschläger and Pörtner 1996; Michaelidis et al. 2005; Pörtner 2008). The latter can limit the energy directed to costly cellular processes such as protein synthesis, resulting in decreased growth and reproduction (Guppy and Withers 1999; Seibel and Walsh 2003).

Echinoderms are abundant marine benthic invertebrates, widely distributed in a variety of habitats, and playing important key roles in their ecosystems. Adults have a poor ability to regulate ion concentration in their extracellular fluids (Stickle and Diehl 1987 and references therein), and they are considered to be hypometabolic (Melzner et al. 2009) as they have low respiratory rates (Lawrence and Lane 1982; Shick 1983). Their oxygen uptake is mostly dependent on the nutritional state, size, and ambient temperature, but also on oxygen tension, seasonality, salinity, and pH (e.g., Hiestand 1940; Farmanfarmaian 1966; McPherson 1968; Sabourin and Stickle 1981; Lawrence and Lane 1982; Brockington and Clarke 2001; Talbot and Lawrence 2002; Siikavuopio and Mortensen 2008; Wood et al. 2008, 2010, 2011; Christensen et al. 2011). The ionic composition of the coelomic fluid is similar to that of seawater, but there are, however, some species whose physiological fluids can be hyperosmotic (Binyon 1966; Ferguson 1990), and there is evidence that limited ionic regulation is possible in some fluid compartments (Binyon 1966; Bishop et al. 1994; Vidolin et al. 2007). The coelomic fluid pH is usually 0.5–1.5 U lower than seawater most likely as a result of CO_2 retention (slow diffusion rate) and due to accumulation of acidic metabolites (Farmanfarmaian 1966; Shick 1983). However, it has been hypothesized that the coelomic fluid has a slightly higher buffer capacity than seawater (Binyon 1966; Shick 1983). Notwithstanding, coelomic fluid pH decreased with seawater pH, indicating either a very low or only partial compensation ability (Miles et al. 2007). These low ion regulation abilities suggest that adult echinoderms could be severely impacted by ocean acidification.

Actually, a variety of responses of adult echinoderms to environmental hypercapnia have been observed and they seem highly species specific. Calcification and/or regeneration of calcified structures was enhanced in a few species submitted to low pH (Wood et al. 2008; Ries et al. 2009), while in others, it was depressed (Gooding et al. 2009; Ries et al. 2009; Wood et al. 2010; 2011). Similarly, effects of low pH on growth differed, both increased and decreased growth rates being reported (Grosjean et al. 1996, 1998; Shirayama and Thornton 2005; Gooding et al. 2009). Feeding rates were reported to be depressed (Siikavuopio et al. 2007). Metabolism and/or oxygen uptake were enhanced at low pH, indicating a higher energetic cost of other function maintenance (Wood et al. 2010, 2011). Finally, temperature effects interacted with those of pH on calcification, growth, and oxygen uptake (Gooding et al. 2009; Wood et al. 2010, 2011; Christensen et al. 2011).

In order to better understand if the metabolic responses of echinoderms were related to their acid–base status, we studied the response of an intertidal sea urchin species, *Paracentrotus lividus*, submitted to low pH at two different temperatures. The pH range was chosen according to near future predictions for ocean acidification, i.e., a decrease of ca. 0.3 to 0.6 U, and the two temperatures (10°C and 16°C) were within the range experienced by this species in the field (Boudouresque and Verlaque 2001). The coelomic fluid of *P. lividus* individuals was characterized and the individual oxygen uptake and the RNA/DNA ratio of gonads were studied as metabolism proxies.

2 Methods

2.1 Experimental setup and procedures

The *P. lividus* individuals were collected by the end of February 2010 during low tide from a temperate European rocky coast in Telgruc-Sur-Mer, Crozon, France. Adults (mean diameter of 36±3.2 mm) were then transported to the laboratory and maintained in aerated seawater until further use (Marine Laboratory of the Brussels University, ULB, Belgium). Experiments took place between March and April 2010. Ten sea urchins per aquaria were submitted to three different pH (8.0—control, 7.7, and 7.4) and to two different temperatures (10°C and 16°C) for 19 days. There were always two replicates (aquaria) per condition (fully crossed design). Low seawater pH were obtained by bubbling CO_2 supplied by Airliquide (France) through electrovalves regulated by a pH controller (Aquastar, IKS ComputerSysteme GmbH, Karlsbad, Germany). All aquaria were kept inside a temperature-controlled room, and they were supplied with ambient air bubbles that originate from outside the room. Aquaria had a 60-L capacity, their water was filtered using semi-dried water pumps (EHEIM, Germany), and seawater renewal was of 50% per week. Individuals were fed artificial sea urchin food (Zeigler^{TM}, USA) *ad libitum*.

2.2 Seawater physicochemical parameters

Salinity was measured using a conductivity meter pH/Cond 340i WTW (USA). The temperature, pH_{NIST} (National Institute of Standards and Technology, previously known as National Bureau of Standards, NBS), and the electromotive force (e.m.f) were measured using a 827 pH Lab Metrohm meter (Switzerland) with a combined glass electrode (Metrohm 6.0228.010 with temperature sensor) calibrated with pH_{NIST} buffers 4 and 7 (Merck CertiPUR®, Germany). The e.m.f values were applied on the calculation of the pH expressed in total scale (pH_T) using standard buffers of known pH, 2-aminopyridine/HCL (AMP) and tris/HCL (TRIS), (DOE 1994; Del Valls and Dickson 1998; Dickson et al. 2007). All reported pH are expressed in seawater scale. Sea water samples were collected and immediately filtered (0.22 μm, Millipore, USA) in order to determine total alkalinity (TA) and magnesium to calcium ratio. The TA was measured by means of a potentiometric titration with HCl 0.1 M using a Titrino 718 STAT Metrohm, and calculated using the Gran function (Gran 1952). Our measurements had a deviation of 0.09% of the standard certified material provided by Andrew G. Dickson's Oceanic Carbon Dioxide Quality Control Laboratory (USA). For Mg^{2+} and Ca^{2+} concentrations used to calculate the magnesium to calcium ratio (Mg/Ca expressed in mol/mol), seawater samples were diluted 20 times in MilliQ water (Millipore) acidified (10%) with HNO_3 65% (Suprapur® Merck, Germany) prior to analyses and were further analyzed with an Iris Advantage (Thermo Jarrell Ash, USA) Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES). The calibration was achieved using artificial multi-elemental solutions made from certified mono-elemental solutions (Merck, Germany) and using seawater certified reference materials (CRM) for quality check (High Purity Standards, USA). Results for the CRM were always within ±10% of the certified values. Dissolved inorganic carbon (DIC), carbon dioxide partial pressure (pCO2), calcite and aragonite saturation state values ($\Omega_{Calcite}$ and $\Omega_{Aragonite}$) were determined from TA, pH_T, temperature, and salinity data using the software CO2SYS (Pierrot et al. 2006) and by using the dissociation constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987) and the K_{SO4} dissociation constant given by Dickson (1990).

2.3 Coelomic fluid

The coelomic fluid (CF) of each individual was collected at the end of the experimental period (16th day) through the peristomial membrane using an insulin syringe (Myjector 0.5 ml, Terumo, Japan). Its e.m.f values were immediately measured using a microelectrode (Metrohm 6.0228.100) whose calibration and conversion to pH in total scale were done as previously described. In addition, CF samples were acidified with HNO_3 65% (Suprapur® Merck), stored at 4°C until ICP-AES analysis where the ion concentrations were measured. Finally, the ΔpH, i.e., the difference between the CF pH_T and the seawater pH_T, was calculated using the mean seawater pH_T.

2.4 RNA to DNA ratio

At the end of the experiments, samples of gonads were collected, frozen in liquid nitrogen, and stored at −80°C until RNA/DNA determinations. Nucleic acid concentrations were determined using a one dye (ethidium bromide)/one-enzyme (RNase), 96-well microplate microplate fluorometric assay based on protocols described by Caldarone et al. (2001) and Belchier et al. (2004) and as reported by Catarino et al. (2008). Samples were analyzed using two replicates to determine total nucleic acid concentration and two for DNA determination after RNA digestion by RNase (R-6513, Sigma-Aldrich) for 30 min at 37°C and 30 min to cool down until room temperature. Ethidium bromide was added to each well, and standard curves were established for each plate using known amounts of 18S- and 28S-rRNA from calf liver (R-0889, Sigma-Aldrich) and ultrapure, highly polymerized calf thymus DNA (D-4764, Sigma-Aldrich). Fluorescence was read using a Spectrofluor Plus microplate reader from Tecan© (Switzerland). Excitation and emission wavelengths were 365 and 590 nm, respectively. The RNA fluorescence was calculated by subtracting the DNA fluorescence reading from the total nucleic acids value. Sample nucleic acid concentrations were estimated by comparing fluorescence readings with those obtained from standard curves. Residual fluorescence (evaluated before the study by using DNase (D-4263, Sigma-Aldrich) was considered to be negligible. The RNA/DNA ratios were determined for each sample and expressed as microgram RNA per milligram dry weight sample divided by microgram DNA per milligram dry weight sample.

2.5 Oxygen uptake

The oxygen uptake was determined for each individual at the 16th day of the experiment in a sealed respirometer cylindrical chamber made of transparent plexiglas, using seawater from the individual aquarium under controlled stirring. Optode oxygen sensors (PreSens, Germany) were attached inside the chamber, and oxygen concentration was measured using a luminescence signal directed and read by means of fiber optics (Fibox 3, PreSens, calibration and salinity corrections were done following manual instructions). Signal is not dependent on flow rate and does not consume oxygen, in opposition to the traditional oxygen electrodes. Measurements were done in temperature-controlled rooms. Oxygen saturation was measured every 10 min for 1 h, and never fell under 80% saturation. Oxygen uptake rate (V_{O2}) was calculated by computing the slope of the linear regression (R^2>0.9) of seawater oxygen content against time. This value was multiplied by water volume and subtracted with the average value for blank incubations. The V_{O2} was expressed in micromoles of uptaken oxygen per gram of individual wet weight per hour.

2.6 Statistical analysis

The level of significance was set at 0.05 for all tests.

Coelomic fluid pH ($\mathrm{pH}_{\mathrm{CF}}$) relation with the mean aquarium pH was studied using regression analysis, first computed at each temperature. The slopes obtained at each temperature were compared using a *t* test (Zar 2005). Taking into account that slopes did not differ between temperatures, a new regression analysis was performed for $\mathrm{pH}_{\mathrm{CF}}$. The ΔpH relation with the mean aquarium pH was studied using Spearman rank correlation (ρ).

The magnesium and calcium concentrations as well as the Mg/Ca ratio in each aquarium were compared using an ANOVA: aquarium (random factor) and temperature (fixed factor). In the coelomic fluid, these parameters were compared using a model III nested ANOVA model (Doncaster and Davey 2007). Factors were pH (fixed), temperature (fixed), and aquarium (random, nested in both pH and temperature). The V_{O2} of the sea urchins was tested using a model III nested ANOVA and using the latter factors.

The individual's sex could not be considered an independent (fully crossed) factor in the RNA/DNA analysis since the urchins were nested within the same treatment (both males and females were present in each aquarium). Another approach was then used, the ratio of the mean effect on females and males within each tank ($[\mathrm{RNA/DNA}]_{\mathrm{F}}/[\mathrm{RNA/DNA}]_{\mathrm{M}}$) was calculated and consequently only one data point was analyzed from each aquarium. A two-way ANOVA was done using as dependent variable the ratio between the RNA/DNA from females and males and using pH and temperature as fixed factors. After verifying, there were no differences on this new ratio between pH and temperature, a *t* test was done to verify if there were differences between the mean RNA/DNA of females and males from each aquaria. To get real independence in the analysis, males from one replicate were compare with the females from another replicate randomly chosen (each aquarium was not repeated in the analysis).

The relationship between biological responses were studied for both sexes at the two different temperatures using the matrix of Pearson's correlation coefficient.

3 Results

3.1 Seawater

Seawater parameters during the experiment are presented on Table 1.

The magnesium and calcium concentrations in seawater did not differ between aquaria of both temperatures (p_{ANOVA} >0.1) and were globally of 58.4±3.06 and 13.1±0.80 mM kg^{-1} (mean ± standard deviation, n=24), respectively. The Mg/Ca ratio also did not differ between aquaria (p_{ANOVA}=0.1) and was 4.47±0.085 (mean ± standard deviation, n=24).

Table 1 Seawater parameters during the experiment (mean ± SD, *n*) (pH in seawater (SW) in total scale)

10°C—nominal pH	7.4		7.7		8.0	
$\mathrm{pH}_{\mathrm{SW}}$	7.40±0.022 (19)	7.40±0.042 (19)	7.67±0.042 (19)	7.69±0.016 (19)	8.00±0.059 (17)	8.01±0.041 (17)
Temperature (°C)	10.4±0.47 (17)	10.3±0.34 (17)	10.7±0.29 (17)	10.5±0.31 (17)	10.4±0.53 (17)	10.4±0.53 (17)
Salinity	32.3±0.19 (17)	32.2±0.09 (17)	32.2±0.14 (17)	32. ± 0.14 (17)	32.2±0.18 (17)	32.1±0.10 (17)
TA (mmol kg^{-1})	2.68±0.096 (7)	2.69±0.082 (7)	2.66±0.055 (7)	2.68±0.065 (7)	2.66±0.062 (7)	2.68±0.048 (7)
DIC (mmol kg^{-1})	2.73	2.74	2.61	2.63	2.30	2.51
$p\mathrm{CO}_2$ (μatm)	2328	2376	1213	1172	529	525
$\Omega_{\mathrm{Calcite}}$	0.93	0.91	1.67	1.73	3.31	3.36
$\Omega_{\mathrm{Aragonite}}$	0.59	0.58	1.06	1.10	2.10	2.12
16°C—nominal pH	7.4		7.7		8.0	
$\mathrm{pH}_{\mathrm{SW}}$	7.39±0.020 (18)	7.40±0.025 (18)	7.67±0.027 (18)	7.70±0.027 (18)	7.90±0.069 (16)	7.92±0.063 (16)
Temperature (°C)	15.8±0.25 (16)	16.0±0.28 (16)	16.1±0.24 (16)	16.0±0.27 (16)	15.9±0.40 (16)	16.0±0.42 (16)
Salinity	32.5±0.18 (16)	32.8±0.21 (16)	32.6±0.19 (16)	32.6±0.24 (16)	32.5±0.19 (16)	32.6±0.20 (16)
TA (μmol kg^{-1})	2.54±0.129 (6)	2.56±0.146 (6)	2.45±0.126 (5)	2.34±0.129 (6)	2.40±0.244 (7)	2.39±0.121 (7)
DIC (mmol kg^{-1})	2.57	2.59	2.38	2.26	2.24	2.22
$p\mathrm{CO}_2$ (μatm)	2364	2330	1135	1012	618	583
$\Omega_{\mathrm{Calcite}}$	1.04	1.09	1.91	1.91	2.97	3.10
$\Omega_{\mathrm{Aragonite}}$	0.67	0.70	1.22	1.22	1.90	1.98

3.2 Coelomic fluid

For each temperature, the coelomic fluid pH ($\mathrm{pH_{CF}}$) decreased with the aquarium seawater pH ($\mathrm{pH_{SW}}$) but showed a large range of variation, $p_{\mathrm{Regression\ 10^{\circ}C}}=1.85\times10^{-3}$, $R^2=0.21$ and $p_{\mathrm{Regression\ 16^{\circ}C}}=0.038$, $R^2=0.082$. As both slopes did not differ (t value$=1.47<t_{0.05(2),89}=1.987$), a single analysis was done using the data from both temperatures, $p_{\mathrm{Regression}}=2.5\times10^{-4}$ and $R^2=0.13$ (Fig. 1). The analysis of the delta pH (ΔpH) showed a significant correlation with the $\mathrm{pH_{SW}}$ ($\rho=0.74$, df=91, $p_{\rho}<2.4\times10^{-17}$).

The Mg/Ca ratio was higher in the coelomic fluid of sea urchins at 16°C (4.6±0.18, $n=59$) treatments than at 10°C (4.4±0.13, $n=56$) ($p_{\mathrm{ANOVA}}=2.1\times10^{-5}$), but did not differ between pH treatments ($p_{\mathrm{ANOVA}}=0.24$). The magnesium and calcium concentrations followed the same trend and were also higher at 16°C than at 10°C ($p_{\mathrm{ANOVA}}<8.0\times10^{-4}$), but did not differ between pH treatments ($p_{\mathrm{ANOVA}}>0.19$). The [$\mathrm{Mg^{2+}}$] were 45.6±2.86 mM $\mathrm{kg^{-1}}$ (mean ± standard deviation, $n=59$) at 16°C and 40.2±4.85 mM $\mathrm{kg^{-1}}$ (mean ± standard deviation, $n=56$) at 10°C, while for [$\mathrm{Ca^{2+}}$], they were 9.7±0.64 mM $\mathrm{kg^{-1}}$ (mean ± standard deviation, $n=59$) and 9.2±1.06 mM $\mathrm{kg^{-1}}$ (mean ± standard deviation, $n=56$), respectively.

3.3 RNA to DNA ratio

The ratio between RNA/DNA from female and male gonads did not differ between pH treatments ($p_{\mathrm{ANOVA}}=0.60$) nor between temperatures ($p_{\mathrm{ANOVA}}=0.25$). The RNA/DNA ratio differed between sex ($p_{\mathrm{t\ test}}=6\times10^{-6}$), females having higher ratios than males. The mean female gonad RNA/DNA ratio was 3.01±1.24 (mean ± standard deviation, $n=55$) and the male one was 0.21±0.17 (mean ± standard deviation, $n=52$).

3.4 Oxygen uptake

The oxygen uptake (V_{O2}) of the sea urchins differed according to pH ($p_{\mathrm{ANOVA}}=2.9\times10^{-2}$) and to temperature ($p_{\mathrm{ANOVA}}=4.0\times10^{-3}$) as well as their interaction ($p_{\mathrm{ANOVA}}=3.1\times10^{-2}$) (Fig. 2). The highest V_{O2} differing from control values was found in individuals submitted to pH 7.7 and 7.4 at 10°C ($p_{\mathrm{Tukey}}=3.48\times10^{-2}$ and 4.86×10^{-2}, respectively) (Fig. 2). The V_{O2} values did not differ according to pH treatment at 16°C and were similar to those of the control at 10°C ($p_{\mathrm{ANOVA}}>6.7\times10^{-2}$).

3.5 Matrix of Pearson's correlation coefficient

The biological responses differed between males and females and also slightly with temperature (Table 2). The RNA/DNA (gonads) response of the females was the least related response with the other variables. The pH of the coelomic fluid ($\mathrm{pH_{CF}}$) was in negatively related with V_{O2} in females, especially at 16°C. In males, both V_{O2} and RNA/DNA (in gonads) were related.

4 Discussion

The coelomic fluid pH ($\mathrm{pH_{CF}}$) of the sea urchin *P. lividus* decreased with the aquarium seawater pH ($\mathrm{pH_{SW}}$), independently of temperature, but this relation only explained 13% of the variation, showing that other more important factors were influencing it. Echinoids have simultaneous an aerobic and anaerobic metabolism under normoxic conditions, due to the low oxygen diffusion into internal tissues (Elligton 1982; Shick 1983; Bookbinder and Shick 1986). The accumulation of organic acidic metabolites, such as lactate, malate, or others might have influenced the pH decrease (Bookbinder and Shick 1986). The delta pH (ΔpH) was lower at lower $\mathrm{pH_{SW}}$, and in the lowest tested pH (7.4), this relation was even negative in some cases, indicating that some individuals can maintain coelomic fluid pH higher than the seawater one. The coelomic fluid pH seems to be compensated in cases of seawater moderate hypercapnia. Its buffer capacity was observed to be higher than that of seawater through titration methods on the sea urchin *P. lividus* (Catarino et al. unpublished) and on other sea urchin species (Gellhorn 1927; Sarch 1932). The coelomic fluid of echinoderms is primarily buffered by the carbon dioxide–

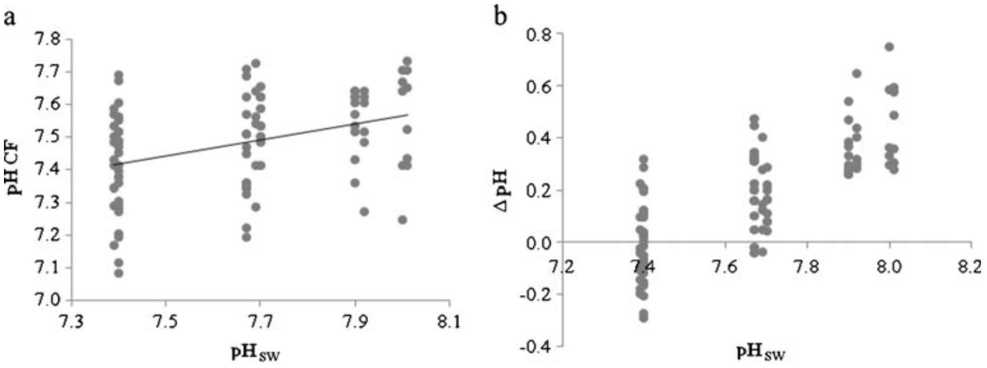

Fig. 1 **a** Coelomic fluid pH (pH CF) according to the aquarium seawater pH ($\mathrm{pH_{SW}}$), $y=0.3\times+5.6$, $p_{\mathrm{Regression}}=2.5\times10^{-4}$ and $R^2=0.13$. **b** Delta pH (Δ pH) according to the $\mathrm{pH_{SW}}$ ($\rho=0.74$, df=91, $p_{\rho}<2.4\times10^{-17}$)

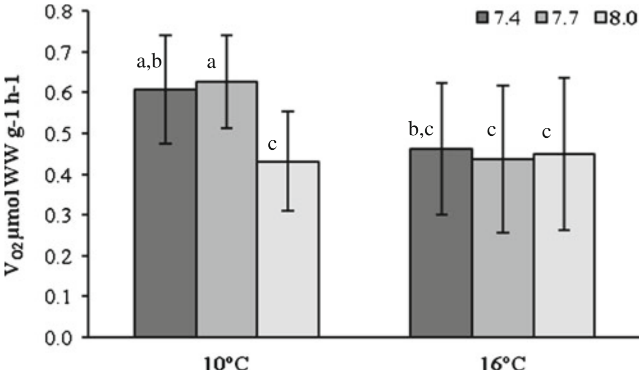

Fig. 2 Mean rates of oxygen uptake (±standard deviation) at the different pH and temperature treatments. *Bars* sharing the same superscript did not differ significantly (α=0.05)

bicarbonate system (Farmanfarmaian 1966; Shick 1983; Miles et al. 2007), but this could be further reinforced by its protein content (0.2–0.5 mg ml^{-1} according to Holland et al. 1967 and Burnett et al. 2002), as hypothesized by Shick (1983), or by other N-containing molecules (Gellhorn 1927). This was further reinforced by a study where the buffer capacity of the coelomic fluid was associated with increased protein concentrations due to ovary growth (Bookbinder and Shick 1986). Even if buffer capacity of such protein concentrations is considered to be low (Heisler 1986; Harrison et al. 1990), the presence of organic and inorganic phosphates, succinate, lactate, ammonia, and other acid or bases produced metabolically and exchanged against strong ions cannot be discarded (Harrison et al. 1990; Truchot 1988; Ali and Nakamura 2000). Our results showed no difference in the magnesium or calcium concentrations of the coelomic fluid, and therefore of the Mg/Ca ratio, between pH treatments. Thus, the nature of the buffer capacity of the coelomic fluid did not seem to be related with increased passive skeleton dissolution, a possible source of HCO_3^-, at lower pH treatments as previously proposed for sea urchins (Spicer et al. 1988; Spicer 1995; Miles et al. 2007).

The Mg/Ca ratio was bigger in the coelomic fluid of sea urchins at 16°C treatments than at 10°C as were the coelomic fluid concentrations of Ca^{2+} and Mg^{2+}. Additionally, ion concentrations were lower than those found in seawater, whose values did not differ between aquaria and temperature. Similarly, Vidolin et al. (2007) observed a lower coelomic fluid [Mg^{2+}] of two sea urchins, *Lytechinus variegatus* and *Arbacia lixula*, when compared with ambient seawater. Despite the fact that sea urchin fluids are globally isosmotic with seawater, the specific ion concentrations are known to vary not only within body compartments but also with the external environment, allowing internal ion gradients to be established (Binyon 1966; Diehl 1986; Stickle and Diehl 1987; Ferguson 1990; Bishop et al. 1994). The fact that at different temperatures ion concentrations varied was most likely related with increased activity of membrane transporters, as it is known to change with temperature (Dowben 1971).

Ion regulation in echinoids, even though limited, is species specific (Binyon 1966) and gains more importance in sea urchins inhabiting coastal or shallow water environments where salinity fluctuations occur (Vidolin et al. 2007). This ability provides individuals with the possibility to resist to osmotic stresses within a limited range (Himmelman et al. 1984; Vidolin et al. 2007). In intertidal environments, pH changes are often related to salinity ones (Truchot and Duhamel-Jouve 1980; Morris and Taylor 1983). The ability to buffer such fluctuations, even if partially, can be an adaptive feature that allows organisms to cope with environmental stresses. For instance, this ability seems to be highly enhanced in crustaceans living in more variable habitats (coastal and/intertidal) and that possess highly improved osmoregulation skills than those which live in more stable environments (Whiteley et al. 2001; Dissanayake et al. 2010). It is known that, for instance, the gastrointestinal epithelium of echinoderms possesses antiporters able to promote proton, ion, and dissolved organic material exchanges with the external milieu (Bamford 1982; Ahearn and Franco 1991; Zhuang et al. 1995). Also, the high density of mitochondria in the intestinal rectum of sea urchins is an indication of its important role in trans-epithelial transport and was linked with ionic regulation (Santos-Gouvea and Freire 2007).

Table 2 Relationship between biological responses studied for both sexes at the two different tested temperatures given by the Pearson correlation matrix

	Females			Males		
10°C	pH_{CF}	V_{O2}	RNA/DNA	pH_{CF}	V_{O2}	RNA/DNA
pH_{CF}	1.00			1.00		
V_{O2}	−0.24	1.00		−0.09	1.00	
RNA/DNA	0.13	−0.10	1.00	−0.20	0.25	1.00
16°C						
pH_{CF}	1.00			1.00		
V_{O2}	−0.60	1.00		0.13	1.00	
RNA/DNA	0.14	−0.03	1.00	−0.06	0.24	1.00

There was an interaction between temperature and pH treatments on the oxygen uptake (V_{O2}) of *P. lividus*. At lower pH (7.4 and 7.7) and at 10°C, the oxygen consumption was higher than in the other treatments (Fig. 2). In echinoderms, the V_{O2} is known to change with temperature (Lawrence and Lane 1982; Shick 1983) and furthermore with pH (Hiestand 1940; Wood et al. 2008, 2010, 2011; Christensen et al. 2011). Interestingly, in our results, the oxygen uptake did not differ between control values from the two tested temperatures. In fact, 10°C and 16°C are temperatures experienced in the field during spring time by the sea urchins *P. lividus* (Boudouresque and Verlaque 2001). Furthermore, in the present study, sea urchins were most likely acclimated to experimental temperatures. Ulbricht and Pritchard (1972) showed that in other echinoid species, when acclimated in laboratory, temperature changes within their tolerance windows did not always led to an increase/decrease of their oxygen consumption. These authors also discussed the fact that metabolic activity in an intertidal species, *Strongylocentrotus droebachiensis*, could be less temperature dependent than in other non-intertidal species, which could also be the case for *P. lividus* intertidal individuals, as in the present study. The feeding activity of sea urchins, can also have an impact on temperature acclimation and consequent variations of metabolic rates (given by oxygen consumption) (McPherson 1968; Lawrence and Lane 1982). In the present study, individuals were fed ad libitum an artificial formula especially made for rearing sea urchins in aquaculture (Zeigler^{TM}), which was therefore highly nutritive. Even if nutrition state seemed optimal, at the lowest temperature and pH, the metabolism was upregulated, indicating a response to an increased energetic demand. Likewise, ophiuroids showed an increase of oxygen uptake when individuals were submitted to lower pH treatments within values predicted for ocean acidification in the near future (Wood et al. 2008, 2010, 2011; Christensen et al. 2011). This energy was most likely used in the maintenance of a normal physiological steady state. At a first stage, this energetic demand did not affect directly other functions such as gonadal growth. Thus, *P. lividus* did not show any difference on RNA/DNA ratio between pH treatments and temperatures. Under normoxic environmental conditions, the gonads of echinoids have a large anaerobic metabolic component (Elligton 1982; Bookbinder and Shick 1986) and so gonad metabolism might not be affected by hypercapnia exposure. Also Lawrence and Lane (1982) reported that ovaries have a higher energetic demand than testis which can represent a higher nutrient drain for the individuals, a fact seen in our results with RNA/DNA being higher in females. Pearson correlation coefficient indicated that in males, gonadal production can be more closely related with the oxidative metabolism.

The RNA/DNA ratio is a reliable indicator of gonadal production (Liyana-Pathirana et al. 2002), and it is known that gonads have both a reproductive and a nutrient storage function (Lawrence and Lane 1982; Hughes et al. 2006). Gonad production depends on food uptake, ingestion rate, reproductive cycle, season, and temperature (Moore 1966; Lawrence and Lane 1982; Liyana-Pathirana et al. 2002; Hughes et al. 2006; Siikavuopio and Mortensen 2008). Some of these factors are deeply related, and their relative contribution can be hard to distinguish. Furthermore, female gonads can even be slightly bigger in some echinoid species, but no differences in feeding rate between sexes have been reported (Lawrence and Lane 1982; Schäfer et al. 2011), implying a considerable complexity of metabolic pathways.

Even though sea urchin coelomic fluid owned a certain buffer capacity, the Pearson correlation coefficient showed a negative relation between the females' pH_{CF} and the individual oxygen uptake (V_{O2}), a measure of energy production, not related directly with the RNA/DNA ratios. So, although ovary production, which can be supported by 76–92% of anaerobic metabolism (Bookbinder and Shick 1986), was not affected by hypercapnia exposure, the metabolic energetic pathways from which it can be dependent, such as nutrient allocation, might have been. In case these pathways depend more directly on extracellular pH, then a higher energetic demand of female individuals could have an indirect impact on aerobic metabolism. On the longer term, gonadal production/growth can be depleted for some sea urchin species. For instance, long-term exposure to pH 7.8 led to a decrease in gonadal development and fecundity of the sea urchin *Hemicentrotus pulcherrimus* (Kurihara unpublished cited in Kurihara 2008 and reviewed by Dupont et al. 2010). Also, sea urchins submitted to severe environmental hypercapnia, during a couple of months, had their gonadal growth affected due to impairment of feeding ability and nutrient conversion efficiency (Siikavuopio et al. 2007). If the energetic demand of the entire organism is kept at abnormal levels for a long period of time or if this is submitted to a more severe pH stress, the individual health may be eventually impaired, especially if nutrient pathways are altered. Since gonad production varies seasonally, these expected differences might be attenuated during some periods of the year.

The sea urchin *P. lividus* presents strategies that allow it to inhabit coastal areas where stress (a parameter that limits production) and disturbance (parameter that causes destruction of biomass) are frequent (Lawrence 1990). Its thermal tolerance window is broad, and it can be exposed to winter temperatures as low as 4°C and summer temperatures as high as 28°C, which suggests a large phenotypic plasticity. In order to better understand acclimation processes of intertidal species such as *P. lividus*, it will be necessary to submitted individuals to hypercapnia on long-term studies, simultaneously exploring their thermal tolerance window.

5 Conclusion

The *P. lividus* pH_{CF} is not mainly ruled by the seawater pH, whereas other parameters are, such as metabolic end products released in the coelomic fluid. These, together with a certain protein and/or phosphoric molecules content, can enhance the buffer capacity of the coelomic fluid, which is considered to be low. The pH_{CF} was not dependent on temperature as well as gonadal production and V_{O2} at control treatments. These facts are most likely related to an acclimation ability of the sea urchin *P. lividus*. The possibility to cope with intertidal seawater parameters fluctuations can be due to a buffer ability of the coelomic fluid, though limited, as well as to a selective control of ion concentrations. Buffer capacity, osmoregulation, and excretion are physiological activities that are intimately related and that could contribute to the maintenance of the metabolic activities of these sea urchins. On the other hand, the metabolism of *P. lividus* was upregulated at lower pH and temperatures. However, only in females the V_{O2} seemed to relate with pH_{CF}. This indicates that a complex energetic pathway might be behind total individual production.

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