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Global warming and ocean acidification affect fertilization and early development of the sea urchin *Paracentrotus lividus*

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Abstract: Increasing values of atmospheric CO_2 and consequent seawater warming, as well as progressive seawater acidification constitute stressors for marine populations that may induce migrations and even local extinctions. We evaluated the separated effects of (1) high predicted temperature levels and (2) low predicted pH values on fertilization and early development stages of the sea urchin *Paracentrotus lividus*. Three treatments of temperature and pH were tested, according to current seawater conditions and values expected for next century in agreement to the IS92a IPCC scenario. We examined fertilization, cleavage rate, and 3-day larvae development at different times after insemination. We found that while warming accelerated fertilization, embryo and larval development, acidification delayed them. These findings suggest contrasting effects of both stressors, which compromise fertilization and early development of *P. lividus* at its southernmost limit of distribution. Further studies about the combined effects of pH and temperature are required to accurately give insight into global warming effects on marine life.

Résumé : Le réchauffement et l'acidification des océans dûs à l'accroissement de la concentration atmosphérique en CO_2 constituent des facteurs de stress pour les populations d'organismes marins qui peuvent induire à des migrations et même à des extinctions locales. Nous avons évalué les effets séparés (1) d'une augmentation de la température et (2) d'une diminution du pH sur la fécondation et le développement précoce de l'oursin *Paracentrotus lividus*. Nous avons testé trois traitements de température et de pH correspondant aux conditions actuelles de l'océan et aux valeurs attendues pour le prochain siècle, d'après le scénario IS92a de l'IPCC. Nous avons examiné le taux de fécondation, le taux de clivage, et le développement de la larve de 3 jours, à différents moments après l'insémination. Nous avons constaté que le réchauffement accélère le succès de la fécondation ainsi que des développements embryonnaire et larvaire alors que l'acidification les retarde. Ces résultats suggèrent que l'acidification et la température ont des effets contrastés compromettant la fécondation et le développement précoce de *P. lividus* à sa limite méridionale de distribution. D'autres études sur les effets combinés du pH et de température sont requises afin de donner un aperçu des effets du réchauffement climatique sur la vie marine.

Keywords: *Paracentrotus lividus* ● Fertilization ● Early development ● Ocean acidification ● Global warming

Introduction

Since the late 19th century, anthropogenic modification of the environment has triggered global climate change processes in which the growth of atmospheric CO_2 concentrations has risen from ~ 280 to 387 ppm (IPCC, 2007). Greenhouse effect has been warming the earth and thus the ocean due to its direct absorption of heat (Harley et al., 2006). When atmospheric CO_2 diffuses into the sea, pCO_2 increases and pH drops down. This reduction leads to a decrease in carbonate ion as well as in aragonite and calcite saturation states, which can affect calcifying marine organisms. According to the IS92a "business as usual" scenario, by the end of the 21st century sea surface temperature (SST) will raise 2-4.5°C, while sea surface pH will fall 0.3-0.4 units (IPCC 2007). Further predictions for the end of the 23rd century talk about a pH fall of ~ 0.7 units (Caldeira & Wicket, 2003; Orr et al., 2005).

Temperature and pH are the most important factors controlling the distribution, physiology, morphology and behavior of marine invertebrates (Doney et al., 2009). In echinoderms, temperature and dissolution of CO_2 modify several physiological processes such as respiration, feeding, fertilization and larval development (e.g. Kurihara & Shirayama, 2004; Dupont & Throrndyke, 2008). During sexual reproduction of many marine invertebrates, such as echinoids, gametes are released to the water column to undertake fertilization, a key process for life and persistence of species. With the ongoing changes in physico-chemical conditions of the ocean, gametes will be discharged into progressively warmer and more acidic waters. Thus, sea urchins gametes are exposed to a variety of stressors, both natural and anthropogenic, that may compromise early stages of development, due to the limited ability to produce calcium structures and apparent low homeostatic capacity (Kurihara & Shirayama, 2004; Melzner et al., 2009). These stages are considered population bottlenecks (e.g. Uthicke et al., 2009) since they determine the size of subsequent stages.

Sea urchins have been long used as models for a range of research topics, wherein testing the effects of temperature and acidification on early stages of development have attracted much attention. Previous studies showed that ocean warming may improve fertilization (Hagström & Hagström, 1959; Mita et al., 1984) and larval dispersal (O'Connor, 2007), but also negative effects on larval development has been documented (Byrne et al., 2011). Adverse effects of acidification on fertilization and early development have also been shown (Kurihara & Shirayama, 2004; Dupont et al., 2008; Havenhand et al., 2008; Clark et al., 2009; Martin et al., 2011; Moulin et al., 2011), with the exception of a study that found no effect of pH over sea urchin fertilization (Byrne et al., 2009). Fewer studies have started to test the combined effects of temperature and pH, with contrasting findings (Byrne et al. 2009, 2010a & b; Sheppard-Brennand et al., 2010).

The sea urchin *Paracentrotus lividus* (Lamarck, 1816) is widespread in the Mediterranean Sea, North Atlantic Ocean and the Canary Islands. The Canary Islands represent the southernmost limit of its distribution, but it is not the region in which the species faces the highest SST: in the Eastern Mediterranean it varies between 13-28°C (Nykjaer, 2009) while in Canaries it ranges 19-25°C (Hernández et al., 2009). This suggests a narrower range of temperature tolerance for the latter population, where the effects of climate change could be highlighted ahead than at higher latitudes. *P. lividus* is both ecological and economically important. The species plays a significant role in rocky intertidal and subtidal ecosystems, since its grazing activity greatly limits macroalgae growth (Girard et al., 2008), but it is also of commercial interest. Thus, studying the impact of climate change on the species' early stages of development can lead to important implications for the functioning of the whole system and for the resource management. In this sense, this study aimed to evaluate the separate effects of predicted values of seawater temperature and pH on fertilization and early stages of development of the most southern *P. lividus* population.

Material and Methods

Physico-chemical seawater parameters

Experiments were conducted with filtered seawater (FSW) purified within a recirculating system provided with DRYDEN AQUA active filter media (AFM) bio-crystals, 10 and 50 μm UNICEL polyamide paper filters and a UV-C AQUAEL 11W filter. We used temperature and pH as stressor factors in two single-factor experiments, each of them with 3 treatments. In order to control seawater temperature and pH (NIST scale), we used a thermostat heater (EHEIM AQUATICS, 50 W) and an Aqua Medic computer bubbling CO_2, respectively. Temperature treatments were: 19°C (control: SST at the Canary Islands), 20.5°C (predicted SST for the year 2050, IPCC, 2007), 22.5°C (predicted SST for 2100, IPCC, 2007). PH treatments were: 8.0 (control: winter seawater pH at the Canary Islands), 7.7 (predicted values for 2100, IPCC, 2007), 7.4 (predicted values for 2300 by the QUIMA group, University of Las Palmas de Gran Canaria). Experiments were conducted in 100 ml pots with FSW at each of the 3 levels of temperature and pH respectively. Water was changed carefully with homemade 40 μm filters once

during the 3-day experiment (at day 2). Monitoring of temperature, pH and salinity of FSW took place 3 times a day during the course of the experiment (n = 9). A 826 pH Mobile meter (Metrohm) with a Primatrode NTC IP pH electrode with temperature sensor was used to measure temperature and pH, while a WTW handheld conductivity meter (COND 315i) was used to measure salinity. Seawater total alkalinity (TA) was calculated for each treatment, according to Lee et al. (2006) for the subtropics in which S: salinity and T: temperature:

$$TA = 2305 + 58.66 \times (S - 35) + 2.32 \times (S - 35)^2 - 1.41 \times (T - 20) + 0.04 \times (T - 20)^2 \quad (1)$$

For each treatment, CO_2 partial pressure (pCO_2), calcite saturation state (Ω_C) and aragonite saturation state (Ω_A) were calculated from TA and pH using the software CO2sys 2011 (Lewis & Wallace, 1998).

Bioassays

Individuals of *P. lividus* were collected in March 2012 from shallow subtidal rocky shores (0-5 m deep) at Boca Cangrejo in Tenerife Island (28°24'N-16°18'W). Sea urchins (mean test size = 45.9 ± 5.9 cm) were induced to spawn by injections of 2-6 ml of KCl into the perivisceral cavity and were placed aboral pole down over beakers with FSW. In order to reduce experimental variability we randomly selected gametes of 4 females and 4 males that were separately mixed before putting gametes of each sex in contact (Evans & Marshall, 2005). To study fertilization and early development 10 ml of eggs and 0.2 ml of sperm (egg:sperm ratio of 1:2400) were put together in pots with 100 ml of FSW with either controlled temperature or pH (3 levels for each factor, n = 4). To study fertilization rate (FR), cleavage rate (CR) and 3-day pluteus larvae development (3LD), pots were incubated 15 minutes, 100 and 200 minutes and 3 days, respectively. After incubation, adding 1 ml of formaldehyde (4%) stopped development. Six aliquots containing 300 randomly selected eggs and/or embryos were observed under a microscope. FR was calculated by means of counting the number of eggs that had a fertilization membrane or exhibited cleavage at each aliquot and then average proportions at each replicate treatment were calculated. Similarly, CR was estimated by the number of eggs in the different stages of cell division at each aliquot: non-fertilized eggs, cells with fertilization membrane, embryos with 2 cells, with 4 cells, and with ≥ 8 cells. To assess 3LD, we took aliquots containing 10-12 randomly selected larvae in each replicate pot, studying a total of at least 40 larvae per treatment in which total larval length (TL), body length (BL) and post-oral arm length (PL) were measured under a microscope (Fig. 1).

Data analysis

Statistical analyses were carried out using PRIMER 6 & PERMANOVA+ v. 1.0.1 software. In order to assess the effects of temperature and pH on FR, data were analysed by 2-way permutational analyses of variance (Anderson, 2001). Two-way repeated measures permutational multivariate analyses of variance (PERMANOVA) (Anderson, 2001) were used to analyze the effects of seawater temperature and pH separately over CR. In each case, 2-way designs were conducted with time after insemination (TAI) (fixed factor; 3 levels for FR: 15, 100 and 200 min, and 2 levels for CR: 100 and 200 min) as the repeated measure testing the treatment effects of either temperature or pH (fixed factors, 3 levels) by sampling period. One-way PERMANOVAs with either temperature or pH as fixed factors were used to analyze morphometric 3LD data. Significant terms were examined using *a posteriori* pairwise comparisons by permutations (Anderson, 2001). If there were not enough possible permutations for a reasonable test, corrected p-values were obtained with Monte Carlo random draws from the asymptotic permutation distribution. Principal Coordinates Analysis (PCO) ordinations of CR and 3LD data were used to identify similarities between observations.

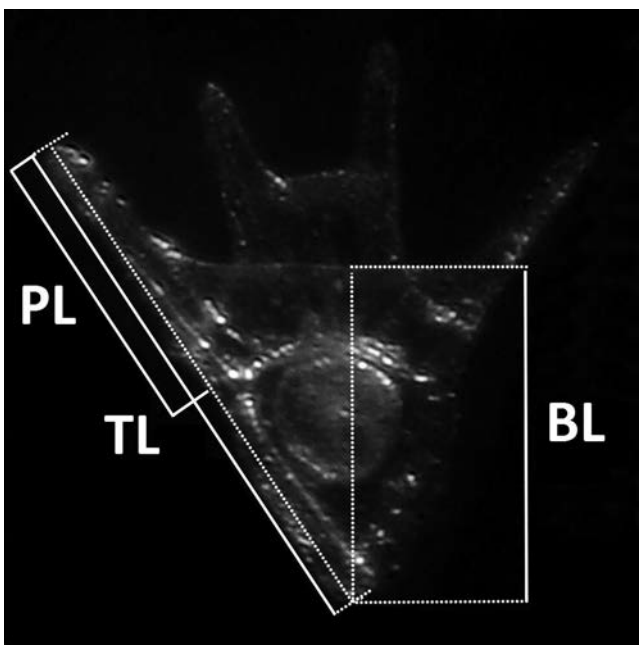


Figure 1. *Paracentrotus lividus.* Morphometric measurements taken on the 3-day 4-arms larvae. TL: total length, BL: body length, PL: post-oral arm length.

Table 1. *Paracentrotus lividus.* Physico-chemical seawater parameters for each treatment. T: Mean temperature, pH: Mean pH, pCO_2: CO_2 partial pressure, S: Mean salinity, TA: Total alkalinity, Ω_C: Saturation level of calcite, Ω_A: Saturation level of aragonite. T, pH and S are expressed with their respective standard deviation.

	Treatment					
	19.0 °C	**20.5 °C**	**22.5 °C**	**pH 7.4**	**pH 7.7**	**pH 8.0**
T (n = 9)	19.17°C ± 0.29	20.41°C ± 0.10	22.4°C ± 0.06	18.75°C ± 0.21	18.70°C ± 0.14	19.00°C ± 0.14
pH (n = 9)	7.97 ± 0.07	8.00 ± 0.07	7.98 ± 0.08	7,44 ± 0,05	7,71 ± 0,04	8,06 ± 0,05
pCO_2	505.4 µatm	464.9 µatm	488.8 µatm	1958.5 µatm	997.8 µatm	396.2 µatm
S (n = 9)	37.93 ± 0.08	37.94 ± 0.07	37.89 ± 0.10	37.6 ± 0.00	37.5 ± 0.07	37.55 ± 0.07
TA (n = 1)	2476,52	2475,46	2467,93	2475.02	2467.54	2474.12
Ω_C	4.22	4.64	4.73	1.29	2.41	4.43
Ω_A	2.74	3.03	3.11	0.84	1.57	2.88

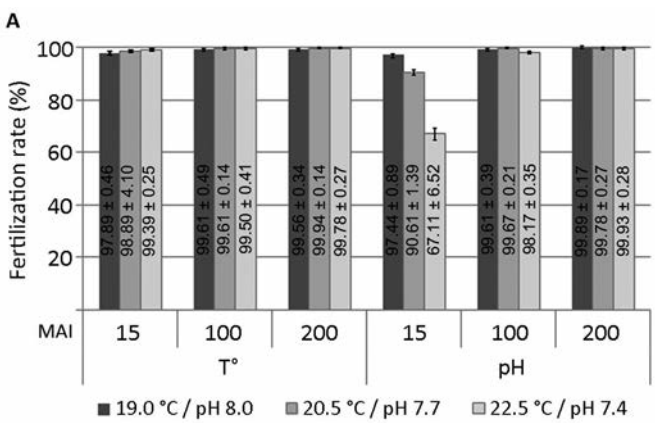
Results

Physico-chemical parameters are given in table 1. Carbon dioxide partial pressure (pCO_2) decreased with temperature rise while it was higher at lower pH. Ω_C and Ω_A increased with temperature and decreased with pH.

High percentages of fertilization (> 97%) were observed in control conditions across time. Significant interactions 'Temperature x Time' and 'pH x Time' were found at each analyses ($F = 9.10$, $p < 0.001$ & $F = 94.95$, $p < 0.001$, respectively). *A posteriori* pairwise analyses revealed that FR significantly decreased with pH, while it slightly but significantly increased with temperature across different times after insemination (Fig. 2). At 15 minutes after insemination (MAI) FR was significantly different between every combination of temperature and pH. At 100 MAI, FR did not differ between temperatures and it was only significantly lower at ph 7.4 than at other treatments. At 200 MAI, there were only significant differences between 19 and 20.5°C, while there were no differences between pH treatments.

Significant interactions 'Temperature x Time' and 'pH x Time' ($F = 396.98$, $p < 0.001$ & $F = 269.82$, $p < 0.001$, respectively), indicate that the influence of temperature and pH on the rate of eggs at different stages of cleavage varied significantly across TAI. Pairwise comparisons showed significant differences between all temperature and pH treatments at both 100 and 200 MAI (Table 2). The higher the seawater temperature, the greater the proportion of eggs in advanced stages of cleavage (Figs 3A & 4A). Contrary, the lower the seawater pH, the greater the proportion of non-cleaved eggs or eggs in early stages of development (Figs 3B & 4B).

Three-day larvae morphology was significantly affected by temperature and pH ($F = 33.8$, $p < 0.001$ & $F = 356.7$, $p < 0.001$, respectively) and it was significantly different



B

Significant results from pair wise test:

Comparisons	t	p-perm
15 MAI 19°C -20.5°C	4.10	0.002
15 MAI 19°C - 22.5°C	7.00	0.001
15 MAI 20.5°C - 22.5°C	2.60	0.021
200 MAI19°C -20.5°C	0.07	0.026

Comparisons	t	p-perm
15 MAI pH8.0 -pH7.7	0.007	0.001
15 MAI pH8.0 -pH7.4	0.003	0.001
15 MAI pH7.4 -pH7.7	0.002	0.001
100 MAI pH8.0 -pH7.4	0.006	0.001
100 MA pH7.4 -pH7.7	0.005	0.001

Figure 2. *Paracentrotus lividus.* **A.** Percentage of fertilized eggs (Mean ± SD, n = 4) at laboratory experiments testing the effects of seawater temperature (19.0, 20.5 & 22.5°C) and seawater pH (8.0, 7.7 & 7.4) at 15, 100 & 200 minutes after insemination (MAI). **B.** Results of pairwise comparisons for significant interactions of "Temperature × time after insemination" and "pH × time after insemination" respectively, found by permutational multivariate analyses of variance (PERMANOVAs). Only significant comparisons are shown. P-perm: p-value using permutations.

Table 2. *Paracentrotus lividus.* Results of pair-wise tests examining the effect of temperature and pH on cleavage rate at laboratory experiments, for significant interaction of factors "Temperature × time after insemination" and "pH × time after insemination" respectively, found by permutational multivariate analyse of variance (PERMANOVAs). Comparisons were performed between levels of temperature and pH for each period of time after insemination studied. MAI: Minutes after fertilization, P-perm: p-value using permutations.

	100 MAI			200 MAI		
	Groups	**t**	**P-perm**	**Groups**	**t**	**P-perm**
Temperature	**19-20.5**	2.14	0.025	**19-20.5**	8.22	< 0.001
	19-22.5	21.06	< 0.001	**19-22.5**	17.43	< 0.001
	20.5-22.5	21.70	< 0.003	**20.5-22.5**	7.11	< 0.001
pH	**8.0-7.4**	22.81	< 0.001	**8.0-7.4**	20.60	< 0.001
	8.0-7.7	31.92	< 0.001	**8.0-7.7**	33.14	< 0.001
	7.4-7.7	13.31	< 0.001	**7.4-7.7**	7.32	< 0.001

across all temperature and pH treatments (Table 3). TL, BL and PL increased with temperature (Fig. 5A) and decreased with pH (Fig. 5B).

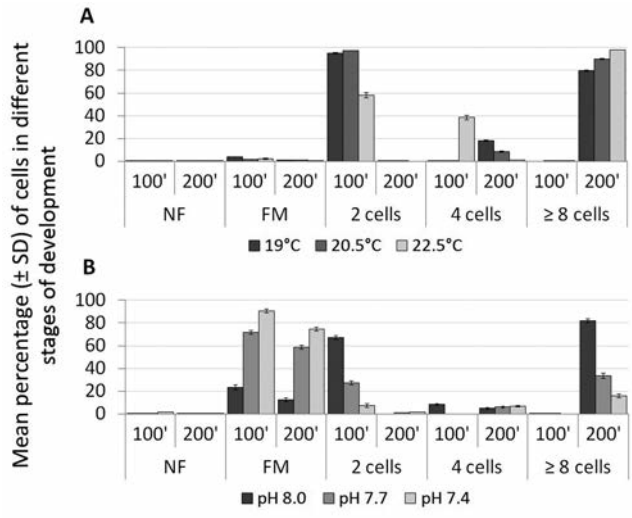


Figure 4. *Paracentrotus lividus.* Mean percentage (± SD, n = 4) of cells in different stages of development at 100 and 200 minutes after fertilization (MAI) according to (**A**) temperature (19.0, 20.5 & 22.5°C) and (**B**) pH (8.0, 7.7 & 7.4) treatments. NF: non-fertilized eggs, FM: eggs only with the fertilization membrane, 2 cells: eggs divided in 2 cells, 4 cells: eggs divided in 4 cells, ≥ 8 cells: eggs divided in 8 or more than 8 cells.

Discussion

Ocean warming effects

We found a high FR (> 97%) (Fig. 2) of the sea urchin *Paracentrotus lividus* at 19-22.5°C, showing that fertilization performance was not only thermo-tolerant but it was even enhanced in warmer waters. High fertilization success (> 95%) has been previously reported for the species at 18 ± 2°C (Privitera et al., 2011), as well as for the tropical species *Heliocidaris erythrogramma* (Valenciennes, 1846) (> 89%) at 20-26°C (Byrne et al., 2009) which, in contrast, displayed a slight but significant decrease of fertilization with increasing temperature. *P. lividus*'s adaptation potential, related to the great variety of habitats and temperature fluctuations that this species copes

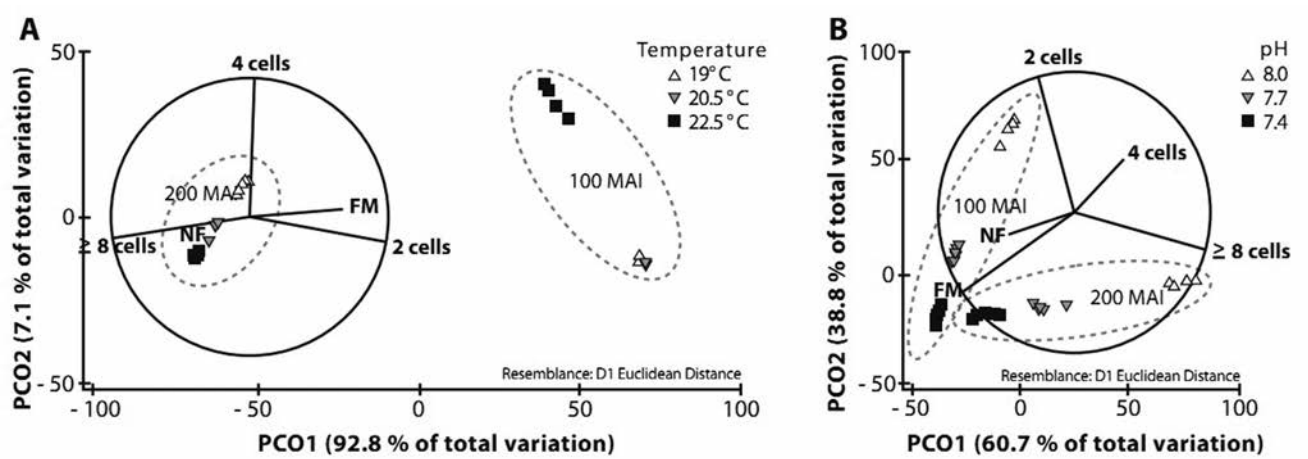


Figure 3. *Paracentrotus lividus.* PCO ordinations showing the effect of both (**A**) seawater temperature (19.0, 20.5 & 22.5°C) and (**B**) pH (8.0, 7.7 & 7.4) on cleavage, at 100 and 200 minutes after insemination (MAI), at laboratory experiments. Vectors represent studied variables. NF: non-fertilized eggs, FM: eggs only with the fertilization membrane, 2 cells: eggs divided in 2 cells, 4 cells: eggs divided in 4 cells, ≥ 8 cells: eggs divided in 8 or more than 8 cells. Percentages of variation explained by each of the axes are given in brackets.

Table 3. *Paracentrotus lividus.* Results of pair-wise tests examining the effect of temperature and pH on larvae morphology within 3 days after insemination at laboratory experiments, for significant factors 'Temperature' and 'pH' respectively, found by permutational multivariate analyses of variance (PERMANOVAs). P-perm: p-value using permutations.

Temperature			pH		
Groups	**t**	**P-perm**	**Groups**	**t**	**P-perm**
19-20.5	5.5	< 0.001	**8.0-7.4**	14.6	< 0.001
19-22.5	9.4	< 0.001	**8.0-7.7**	23.1	< 0.001
20.5-22.5	2.4	0.014	**7.4-7.7**	17.2	< 0.001

by crossing latitudes (Moulin et al., 2011), could be playing an important role in the struggle for survival against climate change. However, fertilization was not dramatically boosted by temperature rise (22.5°C: + 3%) and the observed fertilization acceleration could be just the result of the abatement of fluids viscosity due to water warming, which favors sperm swimming and egg-sperm collision (Hagström & Hagström, 1959; Byrne et al., 2009).

Cleavage speed and embryogenesis, on which depends the subsequent larval development, were high (CR: 95-99 %) and in general slightly rising with temperature (Fig. 4A). At control conditions and 200 MAI ~ 80% of eggs were divided into ≥ 8 cells, while at 20.5 and 22.5°C this proportion increased to ~ 90% and ~ 98%, respectively (Fig. 4A). In contrast, temperature rise to 26°C (pH 8.2) leads to a reduction of 20% on *H. erythrogramma*'s embryos exhibiting normal cleavage (Byrne et al., 2009). Our results evidenced that embryogenesis of *P. lividus* will be favored by temperature rise related to climate change, and will consequently facilitate the eventual transition to the planktonic larval phase.

We found that 3-day larvae exposed to increasing SST showed significant growth of the 3 morphometric variables studied, where the largest growth with respect to the control (57%) was seen in post-oral arms at 22.5°C (Fig. 5A). *P. lividus* populations at the Canary Islands as well as at the Mediterranean, exhibited an accelerated larvae development with increasing temperatures, but competent larvae of the Mediterranean population died within 24 h

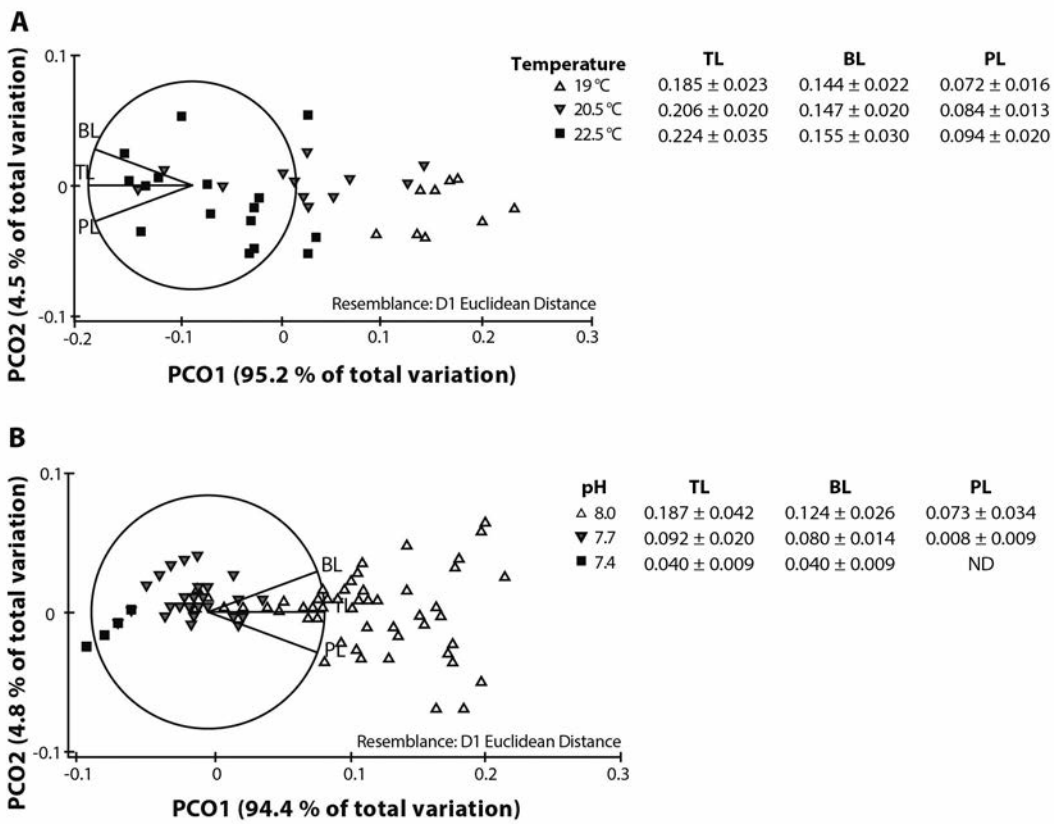


Figure 5. *Paracentrotus lividus.* PCO ordinations showing the effect of both (**A**) sweater temperature and (**B**) pH levels (8.0, 7.7 & 7.4) on larval development within 3 days after insemination at laboratory experiments. Vectors represent studied morphological variables: TL: total length, BL: body length, PL: post-oral arm length. Mean lengths ± SD for each variable are given. ND: non-developed. Percentages of variation explained by each of the axes are given in brackets. Mean larvae sizes ± SD are also reported.

(Privitera et al., 2011). Even if the development of *P. lividus* early stages at the Canaries is boosted by the end of the 21st century, warming could have detrimental effects on latter stages that should be investigated. Although our experiments lasted 3 days, larvae were further kept in culture and they successfully achieved settlement (unpublished data). The observed developmental acceleration with increasing SST reduces the planktonic larval stage, which in turns shortens exposure to predators but also results in smaller dispersion distance (O 'Connor et al., 2007), reducing connectivity among populations (Duarte, 2007). Since a secondary recruitment peak of *P. lividus* has been recorded in Canary Islands with SST > 24°C in summer (Girard et al., 2008), further studies should evaluate the effects of higher temperatures on larvae and recruits and their implications for the dynamics of the species' southernmost population.

In this study we provide for the first time data on the effects of temperature rise on different stages of cleavage as well as on the morphometry of 3-day pluteus larvae from the southernmost *P. lividus* population. We hypothesized that temperature rise would affect more the early development of *P. lividus* Canarian populations which faces higher mean SST through the year and is less adapted to abrupt temperature changes. However, we surprisingly found that this population is robust to temperature rise and that early development is even enhanced at warming conditions.

Ocean acidification effects

Fertilization success of *P. lividus* (> 97%) exposed to acidification was similar to that found by Martin et al. (2011). It was significantly affected by pH: i.e. at 15 MAI, FR decreased 30% at low pH, which is consistent with previous results for the same (Moulin et al. 2011) and other species such as *Hemicentrotus pulcherrimus* Mortensen, 1842 (~ 13%) and *Echinometra mathaei* (Blainville, 1825) (~ 25%) (Kurihara & Shirayama, 2004). In contrast, Martin et al. (2011) and Byrne et al. (2009) did not found significant effects of pH on fertilization of *P. lividus* and *H. pulcherrimus*, respectively. These previous studies used higher temperatures than we did (20 and 20-26°C, respectively) so while hypercapnia's narcotic effect on sperm reduces its motility (Mita et al., 1984), temperature may be having a buffering action in diminishing seawater viscosity and favoring sperm swim (Hagström & Hagström, 1959). Our results suggest that even in the most extreme pH conditions, with standard temperature regimes (19°C), gametes are still viable to reach fertilization and initiate cleavage, despite the significant delay of fertilization. Daily pH fluctuations of about ~ 0.2 units have been reported at shallow coastal areas off the Canary Islands (Hernández, unpublished data), which may explain to some extent *P. lividus*'s tolerance to acidic stress. Likewise, sperm internal pH is about 7.6 units, so gametes can cope with these conditions, which may explain the high FR at pH 7.7 at 15 MAI (90%). Nevertheless, in nature gametes are exposed to a suite of natural factors such as predation and UV-radiation damage that may constrain fertilization success at stressful environmental conditions (Lu & Wu, 2005).

Embryological development was more sensitive to pH stress than fertilization. In control conditions CR was 76 and 87% at 100 and 200 MAI respectively, but at pH 7.4 it significantly decreased 90% (CR_{100MAI} = 8%) and 70% (CR_{200MAI} = 25%) (Fig. 4B). These results could be indicating a narrower range of pH tolerance during cleavage than durind fertilization of *P. lividus*. Moulin et al. (2011) and Kurihara & Shirayama (2004) also found a significant reduction of CR on *P. lividus* (~ 25%) and on *H. pulcherrimus* (~ 37%) at 100 MAI, although abatement of cleavage with acidification was not as dramatic as in our case. At 200 MAI we recorded 82% of eggs divided to more than 8 cells in control conditions (pH = 8.0), but it decreased to 16% at pH 7.4 (~ 80%) (Fig. 4B). In this sense, other species also exhibited a significant reduction in CR, up to ~ 45% in *H. pulcherrimus* (Kurihara & Shiriiyama, 2004). These results clearly demonstrate that acidification delays embryonic development. Such a delay modulate the assortment of embryos able to achieve further development stages, with implications on the subsequent size of each stage and the prolongation of the planktonic phase (O'Connor et al., 2007). Growth and orientation of sea urchin spicules occurs in the transition between amorphous calcium carbonate into calcite in supersaturated waters with respect to $CaCO_3$ (Beniash et al., 1997). When CO_2 diffuses in the ocean, carbonate is converted into bicarbonate by reducing Ω_C and Ω_A due to the excess of H^+ (Clark et al., 2009). We found a depletion of Ω_C and Ω_A with decreasing pH, so as CO_2 dissolves in seawater, the weak transport of CO_2 and bicarbonate ions through the cellular membrane would limit skeletogenesis and post-oral arms growth. This was evidenced mainly on larvae reared at pH 7.4, which showed a 78% reduction of TL and non-development of post-oral arms. Similar studies on the effects of acidification on *P. lividus* larval morphology showed significant reductions of rod size (~ 8%, pH 7.4) (Moulin et al., 2011) and arms length (10-15%, pH 7.5) (Martin et al., 2011). Since post-oral arms of feeding-larvae allow food uptake and swimming capacity (Strathmann, 1971), their delay or lack of development may increase mortality by starvation and susceptibility to predators. This could be exacerbated by the fact that planktonic stages of many echinoids are synchronized with the spring food-bloom and therefore a delay in development may cause a mismatch of the relation food/consumer (Reitzel et al., 2004) with

effects on population dynamics. Even more, variations on larvae survival can translate trough cascades effects (Clark et al., 2009), especially given the role of echinoids as key herbivores, therefore causing changes in the structure and functioning of whole ecosystems.

Moulin et al. (2011) reported negative effects of acidification on *P. lividus*'s early stage of development only for pH < 7.6, but we also found negative effects at pH 7.7. These results could be related to the fact that we used in our experiments parents from the subtidal which are less adapted to abrupt changes of pH. Hence its progeny would have a lower capacity to copes acidification. Contrary to Martin et al. (2011) who also used subtidal parents from the Mediterranean, we found negative effects on FR and larvae morphometry. Thereby, we have reported negative effects of sea water acidification in all studied variables of early stage of development of *P. lividus* populations from the Canary Islands: i.e. on FR, cleavage rate and on 3-day larvae morphology.

Conclusion

The study of seawater warming and acidification as isolated factors in the laboratory, showed antagonistic effects on the fertilization and early development of *P. lividus*; i.e. the former accelerated it, and the later delayed it. While results showed that *P. lividus*'s most southern population, inhabiting subtropical waters characterized by less abrupt oceanographic parameters oscillations, would be tolerant to changes predicted by the end of the 21st century, ocean acidification expected for the 23rd century could have dramatic repercussions. However, these results may not be very realistic since both stressors operate simultaneously in the marine environment, which may result in synergistic, antagonist or additive effects. Further experimentation assessing the interaction of these two factors is needed to clarify their true behavior under more realistic conditions. Intrinsic rhythms of biological processes are crucial for maintaining the viability of populations, but nowadays organisms have to cope with the intricate challenge of inhabit a changing ocean. In this sense, the effects of synergistically interactions between many anthropogenic factors, such as higher UV radiation (due to rupture of the ozone layer), pollution, ocean warming and ocean acidification, must be considered in order to better predict future changes in ecosystems structure, as well as to establish adequate plans of conservation and mitigation.

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