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Thanks mum. Maternal effects in response to ocean acidification of sea urchin larvae at different ecologically relevant temperatures

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ABSTRACT

Juvenile stages of marine species might be more vulnerable than adults to climate change, however larval vulnerability to predictable environmental changes can be mitigated by parental anticipatory buffer effects occurring during gametogenesis. In this study, ocean acidification effect were investigated on larval growth of two sea urchins, *Arbacia lixula* and *Paracentrotus lividus*, at different temperature levels. Results showed that altered pH and temperature affected larval development in both species, with significant length reductions of spicules and significant increases in abnormal larvae. Detrimental effects of reduced pH and high temperature were however dependent on the mother. Furthermore, the responses of *A. lixula* larvae from the ambient site (pH ~ 8.0) were compared with those of larvae obtained from mothers collected from a natural CO_2 vent (pH ~ 7.7) in Ischia. Comparisons highlighted a transgenerational response, as the CO_2 vent larvae proved to be more resilient to reduced pH, although more sensitive to increased temperature.

1. Introduction

Ocean acidification is perceived to be major threat for many species (Sampaio and Rosa, 2019). The absorption of the current increasing atmospheric CO_2 concentration is leading to a progressive reduction in ocean pH (Sabine et al., 2004; Doney et al., 2009), a decrease estimated around 0.2 and 0.4 units by the end of the 21st century (Orr et al., 2005), and by approximately 0.7 units by 2300 if CO_2 emissions will continue at current rates (Zeebe et al., 2008). The measurements carried out by the ALOHA station of the Hawaii Ocean Time-Series (HOT) on the increasing rate of the atmospheric pCO_2 absorbed by the surface waters (Takahashi et al., 2006), indicates that the absorption of anthropogenic CO_2 is the main cause of the long-term increase in dissolved inorganic carbon (DIC) and the decrease in the saturation state of $CaCO_3$. A lower saturation state is linked to the reduction of carbonate ions concentration (CO_3^{2-}), fundamental for biomineralization, with an increment of bicarbonate ions (HCO^{3-}) that are not directly exploitable for organisms (Doney et al., 2012). Hence, the species that use the soluble polymorphs of calcium carbonate (calcite and aragonite) for the construction of skeletons and shells (*i.e.* corals, molluscs and echinoderms) are considered the most susceptible to ocean acidification (Guinotte and Fabry, 2008). Several recent studies have already highlighted the possible negative effects of ocean acidification on cellular parameters related to the immune system (Matozzo et al., 2012; Wang et al., 2015; Castillo et al., 2017; Munari et al., 2019; Munari et al., 2020a), anti-oxidant responses (Moreira et al., 2015; Sui et al., 2017; Munari et al., 2018), growth rates (Bressan et al., 2014; Waldbusser et al., 2015; Kamya et al., 2016; Munari et al., 2016), physiological rates such as clearance, respiration and excretion (Munari et al., 2020b; Asnicar et al., 2021) and reproduction (Cao et al., 2015; Lucey et al., 2015; Munari et al., 2022a) in many marine invertebrates.

To date, most research has focused on the short-term responses of

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individual specimens to individual stressors (Kroeker et al., 2013; Thomsen et al., 2017), while there is still a gap of knowledge about the importance of species capacity for plastic responses and quick adaptation through few generations. Beneficial plastic responses can buffer the negative effects of current climate change (Chakravarti et al., 2016), effectively allowing an organism to maintain its regular functioning and ideally levels of fitness (Hoffmann, 1995; Jarrold et al., 2019). Adaptation occurs as a result of natural selection acting on the genotypic composition of an existing population, in which phenotypic traits are passed on to the next generation. There is growing evidence that the ability to adapt to environmental stressors may depend on the environmental history of the population (Marshall and Morgan, 2011; Asnicar et al., 2021; Vargas et al., 2017, 2022; Munari et al., 2022b) as well as maternal anticipatory effects (Thor and Dupont, 2015). For example, adaptation to environments with high CO_2 concentrations or high CO_2 variability in several marine organisms has been observed (Calosi et al., 2013; Pespeni et al., 2013; Lucey et al., 2016; Teixidó et al., 2020). Nowadays it is pivotal to understand whether marine species will be able to cope with the combined exposure to different environmental changes, and more studies are turning to evaluate whether organisms can achieve beneficial transgenerational plasticity to rapidly adapt to these variations (Byrne et al., 2020; Lee et al., 2020). In a recent study for instance, Munari et al. (2022b) provided first evidence that the tolerance to OA, developed in a polychaetes population living in the CO_2 vent of Castello Aragonese in Ischia (Italy), could affect other important physiological processes and influence the individuals' ability to cope with further stressors.

Generally, the early life stages of marine organisms are more sensitive than adults to environmental changes (Kurihara, 2008; Byrne, 2012; Byrne et al., 2013; Frieder et al., 2014), due to the higher energetic cost for the maintenance of homeostasis with the related reallocation of energy from energy-intensive processes such as somatic growth (Thor and Dupont, 2015). Moreover, many marine species are broadcast spawners, implying that fertilization and then offspring development, occur in the water column, where a significant and prolonged alteration in seawater temperature and pH may directly influence the ecological processes of larval dispersal, growth and survival (Dupont et al., 2010; Brennand et al., 2010; Bestion et al., 2015; McLeod et al., 2015; Crisp et al., 2017). In reproductive structures of marine invertebrates, the inter-individual variation in egg size is often the result of the correlation of egg size with several maternal factors (Bernardo, 1996). The analysis of the eggs size variability in external fertilizers has assumed strong importance in the last decades since it determines the maturity of an efficient zygote. According to Levitan (1993), large eggs are a good target for sperms and increase the fertilization rate, compared to small eggs, thus increasing the fitness of the offspring. Several studies have focused on the combined effects of temperature variations and ocean acidification on female fertility, and most of them found interactive effects of these two stressors, even though there is still a lack of knowledge of the biological consequences of the interactive effects of temperature and ocean acidification on natural populations (Byrne et al., 2009). A study carried out on the sea urchin *Sterechinus neumayeri*, documented a decrease in eggs' size after 6 months of exposure to reduced pH and increased temperature conditions. However, after several months of acclimatization, females of the lower pH treatment produced the largest eggs (Suckling et al., 2015a, b).

Many species of sea urchins are considered keystone species and important grazers that affect the surrounding environment, modifying temporal dynamics and ecosystem functions (Sala et al., 1998; Bonaviri et al., 2012; Hereu et al., 2012; Russell et al., 2012). Since the larval stage, sea urchins are characterized by a high-magnesium calcite skeleton, which is particularly sensitive to lower seawater pH, being 30-times more soluble than calcite (Beniash et al., 1997; Politi et al., 2004). Along the Mediterranean coasts, the echinoids *Arbacia lixula* and *Paracentrotus lividus* play an important role in benthic communities of coastal habitats (Guidetti et al., 2004; Privitera et al., 2005; Gianguzza et al., 2006). In this study we aimed to evaluate the effect of ocean acidification at different levels of temperature on the larval development of two species of sea urchins, *A. lixula* and *P. lividus* living at natural CO_2 vents. Specifically, we sought to isolate the maternal contribution and to assess whether and how much it can guarantee a better response of the larvae to ocean acidification at different levels of temperature within their tolerance window (Boudouresque and Verlaque, 2007; Shpigel et al., 2004; Foo and Byrne, 2021). For this purpose, we used a natural volcanic CO_2 vent, which naturally acidifies the seawater and can be considered a proxy to study the effects of ocean acidification on marine organisms (Hall-Spencer et al., 2008; Foo et al., 2018; Teixidó et al., 2020). The use of organisms that already live under naturally acidified conditions, allows revealing any ability to influence the plasticity of their offspring in response to seawater acidification, compared to embryos developed by gametes of individuals collected in non-acidified areas (ambient-pH conditions).

2. Materials and methods

2.1. Study sites and animal collection

Arbacia lixula and *Paracentrotus lividus* specimens (for both species average diameter test: 46 mm ± SD = 5.7) were collected from subtidal rocky shores, in the northern part of Ischia Island (Tyrrhenian Sea, Italy) between 2 and 5 m depth during what is usually their respective spawning season in the Gulf of Naples (July–August 2018 for *A. lixula*; March–April 2018 for *P. lividus*). In *P. lividus* the length of the spawning season can be influenced by local factors along Mediterranean and Atlantic coasts, usually with its highest peak in mid spring (Lozano et al., 1995; Spirlet et al., 1998; Guettaf et al., 2000; Bayed et al., 2005; Ouréns et al., 2011) with some populations from the Northern Adriatic Sea that can be found fully mature all year around (Munari et al., unpublished data). Regarding *A. lixula*, mature adult specimens can be found all around the year in the Mediterranean Sea (Kempf, 1962; Guidetti and Dulčić, 2007; Wangensteen et al., 2012), although larvae are found to be more abundant in August (Tortonese, 1965). Specifically, *A. lixula* adults were collected in two sampling sites, characterized by different pH conditions (ambient-pH *vs* low-pH conditions). In particular, the site with ambient-pH (~8.0, hereafter 'ambient-pH site') is located at S. Pietro (40° 44′ 47.6″ N; 13° 56′ 40.42″ E), approximately 4 km from the vent site. The site with low-pH (~7.7, hereafter 'vent site') is located around the Castello Aragonese of Ischia (40° 43′ 57.9″ N; 13° 57′ 51.8″ E) and it is characterized by CO_2 emissions that generate a natural pH gradient along the rocky reefs and the *Posidonia oceanica* meadows (Hall-Spencer et al., 2008) (Fig. 1). Regarding *P. lividus*, adults were collected only at ambient-pH site, since at the vent site the size of the population did not allow the collection of enough specimens. The sea urchins were transferred into a flow-through seawater tank at the facilities of the Ischia Marine Centre of the Stazione Zoologica Anton Dohrn. *A. lixula* and *P. lividus* species are not protected or endangered and all experimental procedures on animals were in compliance with the guidelines of the European Union (Directive 609/86). Finally, being the sampling sites located inside the C zone of the Marine Protected Area (MPA) 'Regno di Nettuno', we sampled 30 individuals for *P. lividus* and 30 for *A. lixula*, respectively at the ambient-pH site, and a total of 20 individuals of *A. lixula* at the vent site, following the authorization by the local authorities of the MPA.

2.2. Larval exposure

Adult sea urchins from each population were induced to spawn by injection of 1–2 mL of 0.5 M KCl (Cannavacciuolo et al., 2021). Sperms were collected with a micropipette and kept dry on ice in a plastic tube until use; eggs were collected in 300 mL beakers filled with 0.22 μm filtered seawater (FSW). Before use, eggs and sperms quality and quantity were evaluated using a microscope. To test maternal effects,

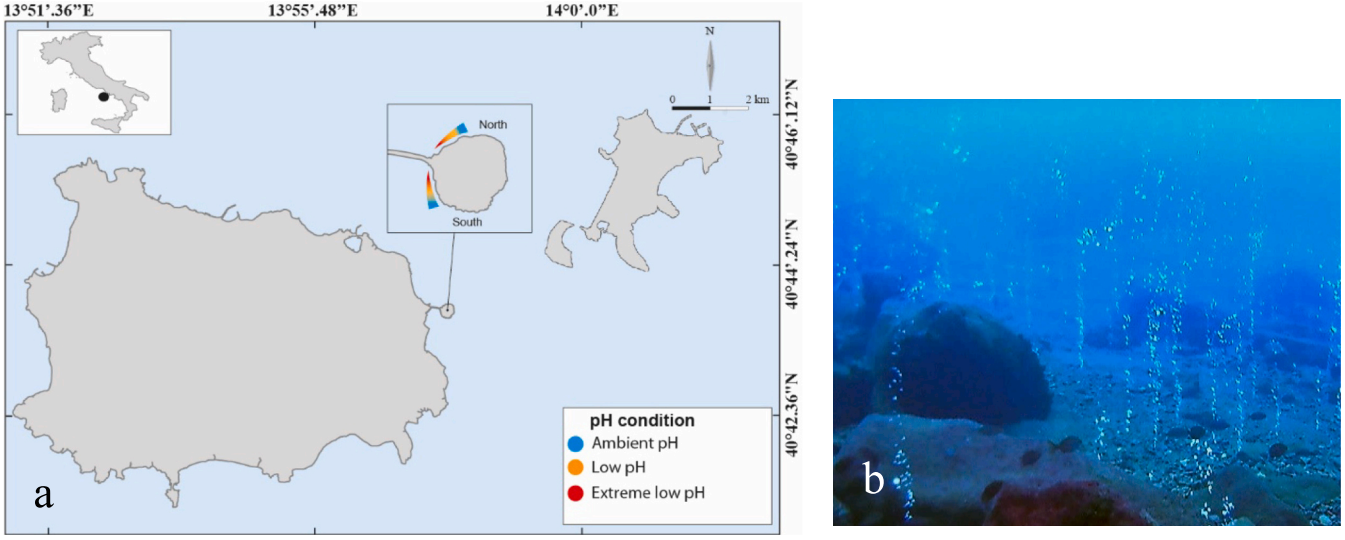

Fig. 1. a) Map showing the location of the CO_2 vent site of the Castello Aragonese with a gradient of pH; b) the underwater volcanic vents occur from 0.5 to 3 m depth, releasing continuously gaseous emissions.
Photo credit: Cristina Palombo.

different families were constituted for both species. It is possible to highlight and estimate parental (both maternal and paternal) sources of variation among offspring of a certain population by using dams and sires factorial designs, such as the North Carolina II cross (Sunday et al., 2011), which consist in creating reciprocal crosses between single males and single females of the experimental population, in order to highlight and quantify the single paternal and maternal contribution in response to a given experimental factor. However, since paternal effects are usually lower than maternal ones, if not even irrelevant, in this study we focus on investigating the maternal contribution in both the species in response to pH at different temperature that can occur during spawning events in geographical areas where this two species can be found.

For *A. lixula* a total number of 10 and 6 families were constituted for the ambient-pH site and the vent site, respectively. For *P. lividus* were constituted 12 families only for the ambient-pH site. To do this, the sperm from each male were counted using a Buerker chamber, then sperm pools were established using the same amount of gametes from each male, to have the same contribution from each father during fertilization. For the experiment with *A. lixula*, pooled sperms from vent site males were used to fertilized only eggs from vent site females, while pooled sperms from ambient-pH site were used to fertilized only eggs from ambient-pH site females. Eggs were collected singularly from each female and fertilized using an aliquot of the pool of sperms. A constant sperm:egg ratio (1250:1) was used (Moschino and Marin, 2002), and fertilization was checked after 30 min by observing the elevation of the jelly coat. All the emissions and the fertilization phases took place at a controlled temperature (22 °C) and ambient-pH conditions. The different number of families was due to the reduced population size of sea urchins from the vent site as well as a higher number of small individuals. Embryo populations were then maintained in closed 25 mL vials (air-tight and completely filled to avoid air exchange that could alter the pH), three replicates for each experimental condition, for 48 h of exposure (Fig. 2). To test the maternal effect in response to the ocean acidification and temperature variations, embryos were exposed to the combination of three different temperatures (16, 22, and 28 °C), and to two different pH conditions (Fig. 2). The pH conditions were chosen considering the average current pH value measured in the ambient-pH site of Ischia (pH_T ~ 8.0, hereafter 'control-pH') and the pH value reflecting the projection under the RCP 8.5 scenario for the year 2100 (pH_T ~ 7.7, hereafter 'low-pH') (Hartin et al., 2016) which is similar to the pH level measured in the vent site (pH_T ~ 7.7). Chosen temperature levels are included in both species tolerance window (Sartori et al., 2015; Foo and Byrne, 2021) and accordingly to the environmental temperatures that can be found during spawning events of both species, along European/African Mediterranean and Atlantic coasts, based on literature and using the geoportal Copernicus (https://cds.climate.copernicus.eu/cdsapp#!/dataset/satellite-sea-surface-temperature?tab=overview). The Copernicus dataset provides global Sea Surface Temperature

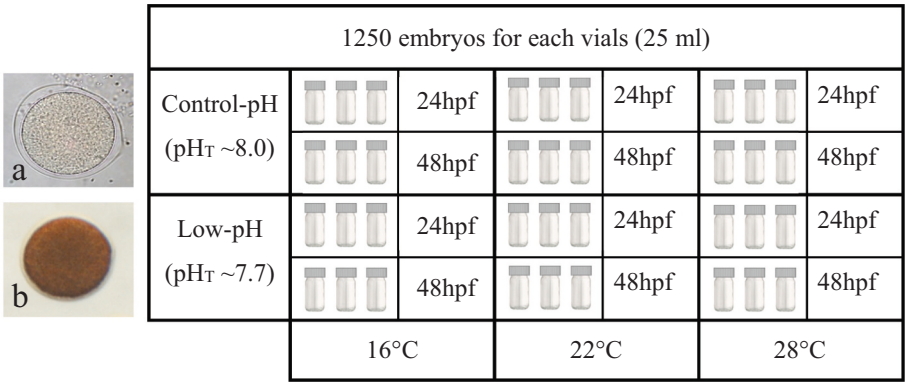

Fig. 2. Experimental design for larval exposure. See the Materials and methods section for details. a) Fertilized eggs of *P. lividus*; b) fertilized eggs of *A. lixula*. 24 hpf: 24 h post fertilization. 48 hpf: 48 h post fertilization.

(SST) data in the raster format (NetCDF) based on multiple sensors with a spatial resolution of 0.05° × 0.05° and no spatial gaps. Daily data of SST from 2013 to 2018 were extracted and monthly average were obtained using the R package 'raster' (Hijmans, 2022). Months for which data were extracted are January, February, April, May, July, August, October and November, being characterized by gamete emission events for both species according to literature. To take into account variety of different spatial context, SST were calculated for the Gulf of Naples (Italy), the Northern Adriatic (Italy), Ancona (Italy), Barcelona (Spain), Porto (Portugal), Atlantic Morocco and Western Algeria (Supplementary Table 1).

This approach has the potential to highlight: (a) if the two species of sea urchins living in the same environment cope differently with the same environmental perturbation; (b) if the offsprings from different parents coming from the same environment are differently susceptible to ocean acidification and different temperatures; (c) if the selective pressure acting during gametogenesis lead to better responses of offspring to pH and temperature changes from adults collected in more stressful conditions (in this case adults with the highest phenotypic plasticity are most likely to spawn and pass down their genotypes to the next generation). This last objective was possible to address only in *A. lixula* as specified in the previous paragraph.

2.3. Data collection

The effects of the three combined factors (warming, pH, and mother of origin) on larval growth were examined at two times post-fertilization (24 h and 48 h). Embryos at 24 h and 48 h post-fertilization (hereafter 'hpf') were observed with an optical microscope (Leica Z16 APO), equipped with a Leica DFC 300FX camera connected to a computer with the Leica LAS program (Leica Application Suite, Version 4.5). A minimum of 100 embryos per replicate were photographed. Different stages of development (Fig. 3a) were distinguished to evaluate any delays in larval development after 24 hpf and 48 hpf of exposure, and the frequencies were calculated. Ontogenetic stages were divided in five groups: 1) arrested embryos, unfertilized eggs, and blastulae; 2) gastrulae, distinguished by the onset of invagination; 3) prisms, when calcareous rods start to form, and the embryo assumes a cuboid form; 4) early plutei, characterized by the four beginning appendixes; and 5) echinoplutei, distinguished for two oral rods and two aboral rods completely formed.

Regarding the echinopluteus stage, the presence of developmental anomalies and differences in growth were evaluated determining the frequency of anomalous larvae and measuring the length of somatic and oral spicules through a program of image analysis (IMAQTM Vision, Version 6.0) (Fig. 3b). As regards the percentage of anomalies and the length of the spicules, only the conditions with a percentage of echinoplutei >20 % (<20 over 100 larvae counted and photographed) were included in the statistical analyses as very low numbers do not allow a correct statistical evaluation of the effects among the experimental conditions tested.

2.4. Statistical analyses

To assess the effects of difference in pH, in temperature, and derived from the maternal effect on larval performances, data were analysed using a non-parametric PERmutational ANalysis Of VAriance (PERMANOVA) test applied on the Euclidean distance matrix (Anderson, 2001). Furthermore, pair-wise *post hoc* tests were carried out once significant differences occurred among and within larval populations at different experimental conditions. PERMANOVA was also used to run pair-wise comparisons among families of the same population to highlight differences in responses of larvae from different mothers. When the number of unique values from permutations was too low, the Monte-Carlo procedure was used to calculate *p* values. Primer software (PRIMER 6.1.10 and Permanova — University of Plymouth, UK) was used for all statistical analyses (Clarke and Gorley, 2006), while graphs were performed with the R software ('ggplot2' package, 4.2.1 R version).

2.5. Carbonate system

Discrete water samples were filtered, collected in borosilicate glass bottles, and immediately fixed with saturated mercuric chloride using standard operating protocols. pH_T (total scale) was determined using an Ocean Optics spectrophotometer (USB2000) with 10 cm path length optical cells and with purified m-cresol purple (Fluidion) (Dickson et al., 2007). Purified m-cresol dye was verified for accuracy compared to tris buffer CRM pH_T (±0.001 SD of spectrophotometer pHT measurements of tris buffer from CRM value) (#26, provided by A. Dickson, Scripps Institution of Oceanography, USA). Water samples were kept in a temperature-controlled water bath (Huber KISS K-12 Refrigerated Heating Bath) at 20 °C before analysis to minimize temperature-induced errors in absorbance measurements. The temperature of each sample was recorded immediately after analysis using a digital thermometer accurate to ±0.05 °C (P600 Dostmann electronic Thermometer). Temperature and salinity (set as constant = 38) were used to calculate the pH_T during the experiment (laboratory conditions) or in the field (CO_2 vent water samples). A_T was determined using an autotitrator (Mettler Toledo G20S). The HCl (0.1 eq. L^{-1}) titrant solution was calibrated against certified reference materials distributed by A.G. Dickson (CRM, Batch #184). The precision of the A_T measurements of CRMs was <4.0 μmol kg^{-1}.

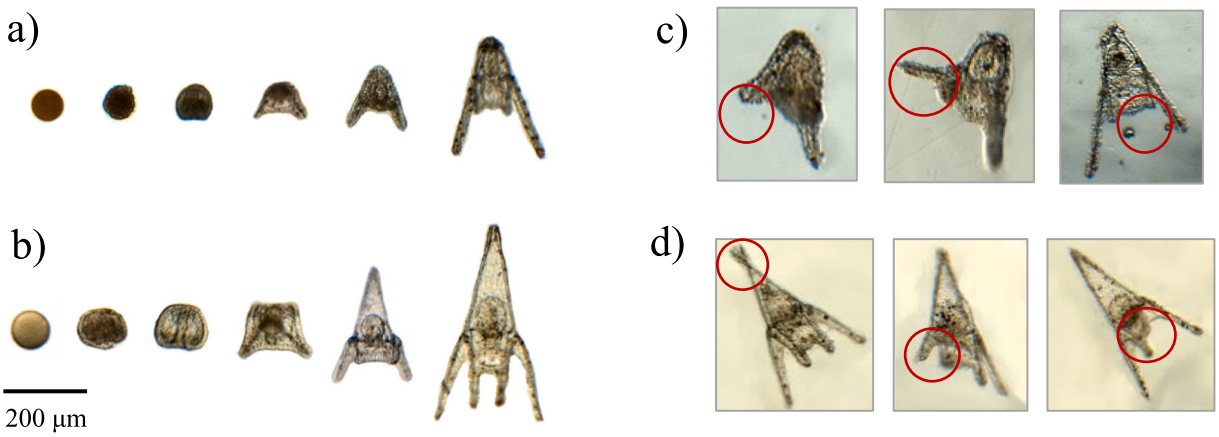

Fig. 3. Developmental stages of *A. lixula* (a) and *P. lividus* (b) during 48 hpf: fertilized egg, blastula, gastrula, prism, early pluteus and echinopluteus (in order from the left). Examples of anomalous echinoplutei of *A. lixula* (c) and *P. lividus* (d) after 48 hpf.

A_T and pH_T were used to determine the remaining carbonate system parameters both in laboratory and field conditions, using the dissociation constants of Dickson (1990) and Dickson and Millero (1987) for $KHSO_4$, and Uppstrom (1974) for boric acid in the R package "seacarb" (Table 1).

3. Results

The PERMANOVA results for larval development parameters measured in *A. lixula* (ambient-pH and vent site) and *P. lividus* (ambient-pH site) larvae throughout the 48 hpf at the different experimental conditions are shown in Table 2. For what it concern larval skeleton development parameters at 48 hpf in *A. lixula* from vent site it was not possible to include in the statistical analyses results obtained from the conditions at 28 °C because of the too low number of echinoplutei founded.

3.1. Larval developmental stages

After 24 hpf, a significant effect of pH * temperature * family interaction was evident in larval development in all three experimental populations (Table 2). In particular, the main driving factors were temperature and mother of origin as highlighted by PERMANOVA (Table 2). As shown in Fig. 4a, at 16 °C the development of both species (*A. lixula* and *P. lividus*) fails to overcome the prism stage. On the contrary, at 22 and 28 °C embryos reached the early pluteus stage at both pH conditions, even if a significant difference in early plutei percentage between the two temperatures was observed in the three populations (Fig. 4a). An opposite trend was observed between the two species from the ambient-pH site, with a percentage of early plutei of 62.4 % at 22 °C and 75 % at 28 °C in *A. lixula*, while in *P. lividus* early pluteus percentages reached 82.4 % at 22 °C and 66 % at 28 °C. Conversely to embryos of *A. lixula* coming from the ambient-pH site, those from the vent site developed better at 22 °C with a percentage of 69.23 of early plutei while at 28 °C early plutei were only 5.47 %.

This temperature related-trend was observed at both pH conditions for all the three populations tested. Overall, a significant maternal effect was observed in *A. lixula* (from both ambient-pH and vent sites) and *P. lividus* in response to experimental conditions. Despite this, the magnitude of this effect was different between the embryos obtained from the different populations, as highlighted by the variability of the response to temperature among families (Figs. S1, S2, S3).

Accordingly to 24 hpf, after 48 hpf a significant effect of pH * temperature * family interaction was detected in both species from the ambient-pH site (Table 2). In particular, for *A. lixula* from the ambient-pH site all the three factors were determinant for embryos development, while for *P. lividus* only the factors temperature and family were significantly different. As shown in Fig. 4b, the lower temperature significantly slowed down the development, with the total absence of echinoplutei at 16 °C for all the three experimental populations. At 22 and 28 °C, embryos of both species from each site reached the echinopluteus stage at both pH levels even if the percentage was very low for the vent site *A. lixula* population.

In *A. lixula* from the ambient-pH site, no differences were observed among the echinoplutei grown at 22 °C and 28 °C while there was a trend due to reduced pH (Fig. 4b) with a higher average percentage of echinoplutei at control-pH (69.12 %) compared to low-pH (55.36 %). *A. lixula* population from the vent site was significantly affected by the highest temperature, resulting in an average percentage of echinoplutei of 89.84 % at 22 °C and 6.59 % at 28 °C. Regarding *P. lividus* there was a trend in response to temperature with a higher average percentage of echinoplutei at 22 °C (95.33 %) compared to 28 °C (81.59 %) at both pH levels. After 48 hpf, a significant maternal effect was observed in response to experimental conditions for all three experimental populations. Also in this case the magnitude of this effect was different between the embryos obtained from the different populations, as highlighted by the variability of the response to temperature among families (Figs. S4, S5, S6).

3.2. Echinoplutei skeletal abnormalities

After 48 hpf, there was not a significant pH * temperature * family interaction in *A. lixula* larvae from the ambient-pH site, while the normal larval growth of *P. lividus* echinoplutei was found to be sensitive to the interaction of the three experimental factors (Table 2, Fig. 5). For the ambient-pH site *A. lixula* population, the main factors influencing the normal development were pH and family of origin. In particular, the average percentage of abnormal echinoplutei at control-pH was 34.50 % while at low-pH was 48.00 %. For the vent site *A. lixula* population, abnormal larval development was affected by both pH and family of origin but not by their interaction, with a reduced percentage of abnormal echinoplutei at low-pH (36 %) compared to larvae reared at control-pH (23 %) (Fig. 5). Regarding *P. lividus*, larvae showed to be affected by temperature and family as well, with an average percentage between the two pH levels of abnormal echinoplutei of 16 % at 22 °C and 52 % at 28 °C (Fig. 5).

In both populations of *A. lixula* and *P. lividus* from the ambient-pH site the interactions of the mother of origin with both pH and temperature were significant (Table 2). The variability of the response among families regarding the temperature was similar between the two species coming from the ambient-pH site with the SD of the transformed (square root) average % of normal echinoplutei kept at 22° about 1.25 for *A. lixula* and 0.78 for *P. lividus* while at 28 °C was respectively 1.76 and 1.05. The same trend of variation between the two ambient-pH site populations was observed also with pH, with the SD among families of the transformed (square root) average % of abnormal echinoplutei kept at control-pH about 1.74 in *A. lixula* and 1.01 in *P. lividus* while at low-pH was 1.51 and 0.79 respectively. At 22 °C, an opposite trend of variation among families was observed in vent site *A. lixula* when exposed to the two pH levels, with an SD of about 0.89 at control-pH and 0.92 at low-pH.

The length of both SSL (define) and OSL (define) was also affected by the interactions between mother of origin with both temperature and pH for *A. lixula* from the ambient-pH site (Table 2). The same trend of response against pH was observed at both temperatures with an average reduction of spicules length of about 5.03 μm for SSL and of about 24.15 μm for OSL (Fig. 6). In *A. lixula* from vent site, the mother of origin significantly influenced both SSL and OSL spicules while reduced pH

Table 1
Measured and estimated seawater physiochemical parameters (mean ± SD, n = 3 for each experimental conditions) at laboratory experimental conditions and in the field (CO_2 vent site) for pH_T, total alkalinity (A_T), dissolved inorganic carbon (DIC), pCO_2, calcite (Ωc) and aragonite (Ωa) saturation states. T, pH_T, and A_T were measured and the other parameters (C_T, pCO_2, Ωc, and Ωa) were estimated by using the R package "seacarb".

	Experiment conditions	pH_T	A_T (μmol kg^{-1})	DIC (μmol kg^{-1})	pCO_2 (μatm)	Ωa	Ωc
Arbacia lixula	Ambient-pH lab	8.04 ± 0.01	2664.71 ± 73.02	2314.60 ± 69.70	463.90 ± 35.79	3.98 ± 0.14	6.01 ± 0.18
	Low-pH lab	7.68 ± 0.02	2709.15 ± 18.52	2562.78 ± 45.01	1273.85 ± 169.41	2.02 ± 0.27	3.05 ± 0.39
	pH vent	7.66 ± 0.06	2588.54 ± 5.11	2431.62 ± 4.90	1244.92 ± 2.51	2.10 ± 0.00	3.15 ± 0.01
Paracentrotus lividus	Ambient-pH lab	8.04 ± 0.04	2635.19 ± 2.07	2208.59 ± 1.83	377.37 ± 0.31	4.83 ± 0.00	7.24 ± 0.01
	Low-pH lab	7.67 ± 0.02	2631.78 ± 24.03	2453.81 ± 22.93	1141.02 ± 10.66	2.32 ± 0.02	3.47 ± 0.03

Table 2
PERMANOVA results. Pseudo-F values (indicated as F) and Monte Carlo *p*-values (indicated as P_{MC}) regarding the developmental stages at 24 and 48 hpf, the anomalies and the length of somatic and oral spicules of larvae coming from ambient-pH site (pHT ~ 8.0) adults and vent site (low-pH, pHT ~ 7.7) adults for all the experimental factors and their interactions (control-pH — pHT ~ 8.0, low-pH — pHT ~ 7.7; temperatures 16, 22, 28 °C).

	Developmental stages 24 hpf			Developmental stages 48 hpf			Abnormal larvae 48 hpf			Somatic spicules length 48 hpf			Oral spicules length 48 hpf		
	A. lixula ambient-pH site	*A. lixula* vent site	*P. lividus* ambient-pH site	*A. lixula* ambient-pH site	*A. lixula* vent site	*P. lividus* ambient-pH site	*A. lixula* ambient-pH site	*A. lixula* vent site	*P. lividus* ambient-pH site	*A. lixula* ambient-pH site	*A. lixula* vent site	*P. lividus* ambient-pH site	*A. lixula* ambient-pH site	*A. lixula* vent site	*P. lividus* ambient-pH site
Family, Fa	**$F_{(9,120)}$ = 12.626 P_{MC} < 0.001**	**$F_{(5,72)}$ = 19.372 P_{MC} < 0.001**	**$F_{(11,144)}$ = 29.966 P_{MC} < 0.001**	**$F_{(9,120)}$ = 12.729 P_{MC} < 0.001**	**$F_{(5,72)}$ = 5.936 P_{MC} < 0.001**	**$F_{(11,144)}$ = 12.938 P_{MC} < 0.001**	**$F_{(9,80)}$ = 59.224 P_{MC} < 0.001**	**$F_{(5,24)}$ = 4.564 P_{MC} < 0.001**	**$F_{(11,96)}$ = 24.002 P_{MC} < 0.001**	**$F_{(9,80)}$ = 155.01 P_{MC} < 0.001**	**$F_{(5,24)}$ = 4.417 P_{MC} = 0.006**	**$F_{(11,96)}$ = 11.549 P_{MC} < 0.001**	**$F_{(9,80)}$=44.448 P_{MC} < 0.001**	**$F_{(5,24)}$ = 11.747 P_{MC} < 0.001**	**$F_{(11,96)}$ = 9.572 P_{MC} < 0.001**
pH	$F_{(1,120)}$ = 1.691 P_{MC} = 0.209	$F_{(1,72)}$ = 0.194 P_{MC} = 0.876	$F_{(1,144)}$ = 1.517 P_{MC} = 0.234	**$F_{(1,120)}$ = 4.078 P_{MC} = 0.028**	$F_{(1,72)}$ = 1.239 P_{MC} = 0.323	$F_{(1,144)}$ = 0.942 P_{MC} = 0.368	**$F_{(1,80)}$ = 7.480 P_{MC} = 0.023**	**$F_{(1,24)}$ = 8.488 P_{MC} = 0.034**	$F_{(1,96)}$ = 2.562 P_{MC} = 0.133	$F_{(1,80)}$ = 4.067 P_{MC} = 0.075	$F_{(1,24)}$ = 1.104 P_{MC} = 0.342	$F_{(1,96)}$ = 4.0529 P_{MC} = 0.072	**F(1,80) = 9.109 P_{MC} = 0.015**	**$F_{(1,24)}$ = 35.313 P_{MC} = 0.002**	$F_{(1,96)}$ = 0.109 P_{MC} = 0.747
T (°C)	**$F_{(2,120)}$ = 117.010 P_{MC} < 0.001**	**$F_{(2,72)}$ = 52.642 P_{MC} < 0.001**	**$F_{(2,144)}$ = 137.890 P_{MC} < 0.001**	**$F_{(2,120)}$ = 50.421 P_{MC} < 0.001**	**$F_{(2,72)}$ = 37.334 P_{MC} < 0.001**	**$F_{(2,144)}$ = 205.530 P_{MC} < 0.001**	$F_{(1,80)}$ = 4.321 P_{MC} = 0.065		**$F_{(1,96)}$ = 67.564 P_{MC} < 0.001**	$F_{(1,80)}$ = 5.254E-02 P_{MC} = 0.824		$F_{(1,96)}$ = 7.981 P_{MC} = 0.016	$F_{(1,80)}$ = 0.454 P_{MC} = 0.514		**$F_{(1,96)}$ = 28.592 P_{MC} < 0.001**
Fa * pH	**$F_{(9,120)}$ = 4.849 P_{MC} < 0.001**	$F_{(5,72)}$ = 1.291 P_{MC} = 0.227	**$F_{(11,144)}$ = 1.725 P_{MC} = 0.034**	**$F_{(9,120)}$ = 5.879 P_{MC} < 0.001**	$F_{(5,72)}$ = 1.366 P_{MC} = 0.161	**$F_{(11,144)}$ = 2.148 P_{MC} = 0.008**	**$F_{(9,80)}$ = 10.526 P_{MC} < 0.001**	$F_{(5,24)}$ = 2.255 P_{MC} = 0.079	**$F_{(11,96)}$ = 6.637 P_{MC} < 0.001**	**$F_{(9,80)}$ = 7.245 P_{MC} < 0.001**	$F_{(5,24)}$ = 2.090 P_{MC} = 0.101	**$F_{(11,96)}$ = 3.1432 PMC = 0.0011**	**$F_{(9,80)}$ = 14.008 P_{MC} < 0.001**	$F_{(5,24)}$ = 0.893 P_{MC} = 0.499	**$F_{(11,96)}$ = 3.260 P_{MC} < 0.001**
Fa * T	**$F_{(18,120)}$ = 7.470 P_{MC} < 0.001**	**$F_{(10,72)}$ = 13.560 P_{MC} < 0.001**	**$F_{(22,144)}$ = 26.863 P_{MC} < 0.001**	**$F_{(18,120)}$ = 6.218 P_{MC} < 0.001**	**$F_{(10,72)}$ = 5.522 P_{MC} < 0.001**	**$F_{(22,144)}$ = 13.470 P_{MC} < 0.001**	**$F_{(9,80)}$ = 6.952 P_{MC} < 0.001**		**$F_{(11,96)}$ = 16.722 P_{MC} < 0.001**	**$F_{(9,80)}$ = 11.232 P_{MC} < 0.001**		**F(11,96) = 24.863 P_{MC} < 0.001**	**$F_{(9,80)}$ = 5.961 P_{MC} < 0.001**		**$F_{(11,96)}$ = 17.330 P_{MC} < 0.001**
pH * T	$F_{(2,120)}$ = 0.764 P_{MC} = 0.572	$F_{(2,72)}$ = 1.283 P_{MC} = 0.293	$F_{(2,144)}$ = 1.252 P_{MC} = 0.299	**$F_{(2,120)}$ = 6.243 P_{MC} = 0.001**	$F_{(2,72)}$ = 1.383 P_{MC} = 0.248	$F_{(2,144)}$ = 1.239 P_{MC} = 0.293	$F_{(1,80)}$ = 1.849 P_{MC} = 0.205		$F_{(1,96)}$ = 0.973 P_{MC} = 0.342	$F_{(1,80)}$ = 4.373 P_{MC} = 0.067		$F_{(1,96)}$ = 2.38E−03 P_{MC} = 0.959	$F_{(1,80)}$ = 0.944 P_{MC} = 0.358		$F_{(1,96)}$ = 1.574 P_{MC} = 0.243
Fa * pH * T	**$F_{(18,120)}$ = 4.676 P_{MC} < 0.001**	**$F_{(10,72)}$ = 1.965 P_{MC} = 0.013**	**$F_{(22,144)}$ = 2.319 P_{MC} < 0.001**	**$F_{(18,120)}$ = 4.054 P_{MC} < 0.001**	$F_{(10,72)}$ = 1.387 P_{MC} = 0.096	**$F_{(22,144)}$ = 1.984 P_{MC} = 0.002**	$F_{(9,80)}$ = 1.114 P_{MC} = 0.366		**$F_{(11,96)}$ = 5.157 P_{MC} < 0.001**	$F_{(9,80)}$ = 1.291 P_{MC} = 0.253		**$F_{(11,96)}$ = 2.634 P_{MC} = 0.007**	$F_{(9,80)}$ = 1.875 P_{MC} = 0.068		**$F_{(11,96)}$ = 2.003 P_{MC} = 0.041**

Significant results are in bold.

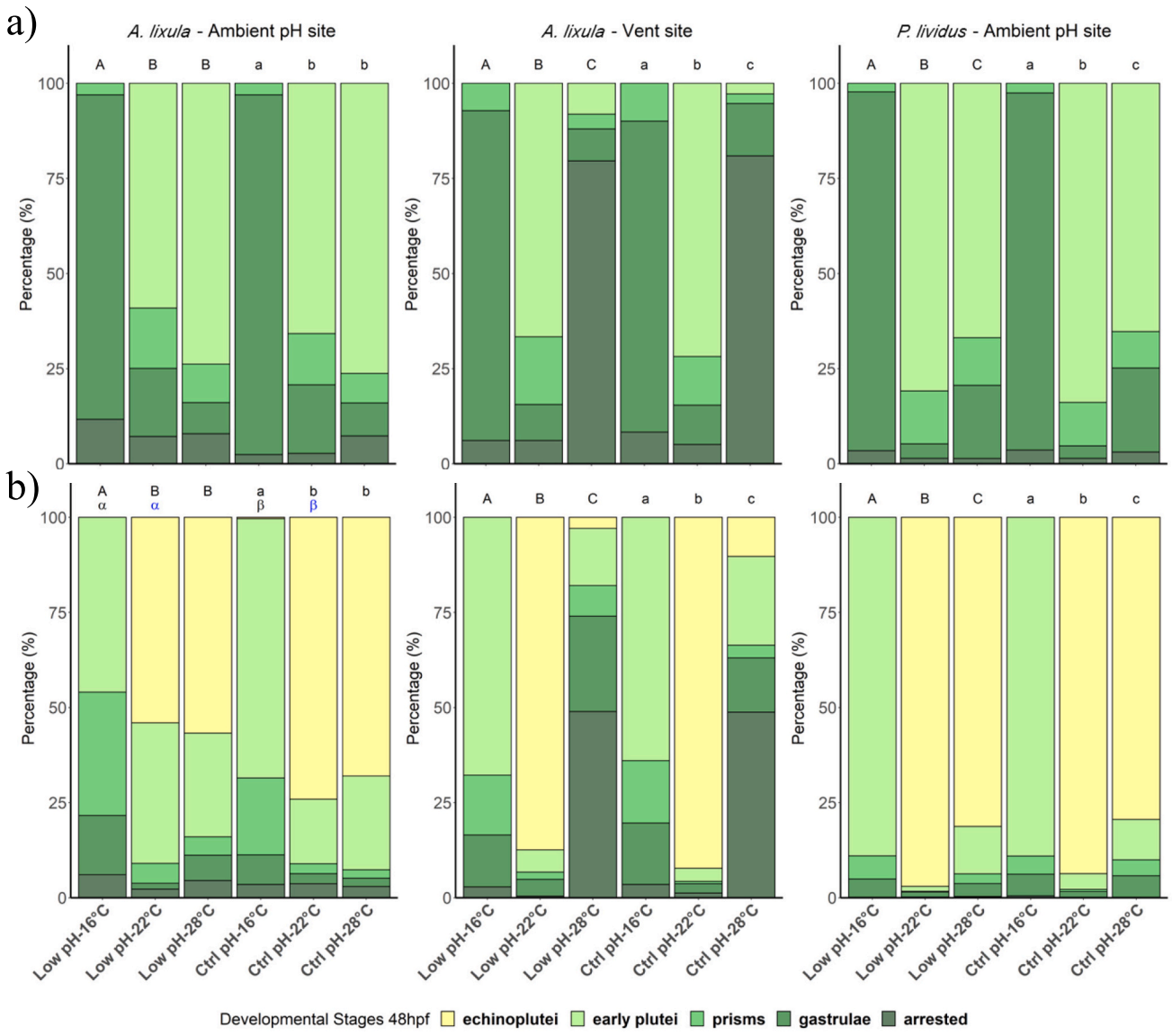

Fig. 4. Percentage of developmental stages at 24 hpf (a) and 48 hpf (b). On the X-axis the larval exposure conditions are shown. Significant development differences ($p < 0.05$) for low-pH conditions ($pH_T \sim 7.7$) are represented with capital letters (A–C), while lower-case letters (a–c) were used for control-pH conditions ($pH_T \sim 8.0$).

significantly influenced only OSL with an average reduction among families of about 7.31 μm at 22 °C (Figs. S10, S11, S12).

Both SSL and OSL in *P. lividus* were significantly influenced by the pH * temperature * family interaction (Table 2). In particular, the length of both types of spicules was significantly reduced by increased temperature with an average difference between larvae kept at 22 °c and 28 °C of about 21.81 μm for SSL and 39.72 μm for OSL (Fig. 6).

In ambient-pH site *A-lixula* the variation in spicules length among families was greater with increased temperature, for both SSL and OSL. On the contrary, in response to pH, the variation among families was greater at control-pH for both SSL and OSL. The same trend was observed in *P. lividus* with a greater variation in spicules length among families at the highest temperature for both spicules kind. Also in response to pH, the variation among families of *P. lividus* was greater at control-pH.

At 22 °C, the same trend of variation among families was observed in vent site *A. lixula* when exposed to the two pH levels, with a higher variability at control-pH compared to low-pH for both SSL and OSL.

4. Discussion

The combined effects of ocean acidification and increased temperature may exacerbate the detrimental effects on marine species (*i.e.* sea urchins) that are characterized by pelagic larval stages that develop calcareous structures since the early life stages, making them particularly susceptible to variations of abiotic conditions in the water column (Byrne, 2012; Doney et al., 2012).

Evaluating the impact of environmental stressors on the early life stages is therefore clearly important since they represent a possible bottleneck for the maintenance of natural populations (Dupont et al., 2010; Byrne, 2012). However, vulnerability to environmental perturbations may be mitigated by some maternal anticipatory effects (Marshall and Uller, 2007). To tackle these issues, this study investigated if (i) the larval development of *Arbacia lixula* and *Paracentrotus lividus* was differently susceptible to the induced stressors (temperature alterations and pH), (ii) if the pattern of developmental larval response was different between the two *A. lixula* populations (ambient-pH *vs* vent sites) and (iii) if a maternal effect was highlighted in response to a variance of pH and temperature in all the three experimental

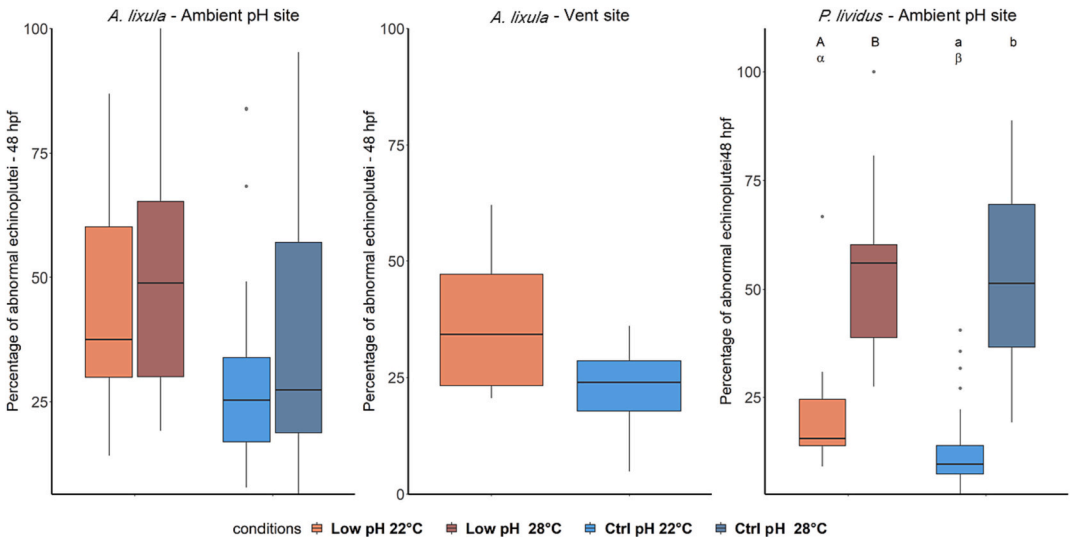

Fig. 5. Percentage ± SD (n = 3) of abnormal echinoplutei at 48 hpf (A). Mean length ± SD (n = 3) of somatic spicules at 48 hpf (B). On the X-axis the larval exposure conditions are shown. Significant differences ($p < 0.05$) among temperatures at low-pH ($pH_T \sim 7.7$) are represented with capital letters (A–C), while lower-case letters (a–c) were used for control-pH (~8.0).

populations.

4.1. Thermal and low pH responses of larval development and growth

In accordance with the general knowledge about the effect of low pH on echinoderms' larvae (Byrne et al., 2013; Foo and Byrne, 2021) we found reduced growth and calcification in all the larval populations of both species eventhough, altogether, *A. lixula* generally showed to be more influenced by low pH than *P. lividus*, regardless of the temperature, especially in larvae obtain from ambient-pH adults. Species that have a large larval distribution such as *A. lixula* (Lessios et al., 2012; Wangensteen et al., 2012) seem to be able to better cope with ocean warming compared to others with a similar larval dispersion (Hardy et al., 2014) as highlighted from our results from the ambient-pH population when compared with *P. lividus*. Indeed, *P. lividus* has instead a narrow range of thermal tolerance in the different populations (18–22 °C), although it also has a wide geographical distribution in the Mediterranean Sea (Boudouresque and Verlaque, 2001).

Different populations of *A. lixula* can have different thermal tolerances during their development, linked to the Mediterranean region where they come from (Pecorino et al., 2013; Hardy et al., 2014; Byrne et al., 2016). For instance, Wangensteen et al. (2013a, b) found that larvae of *A. lixula* were able to develop at temperatures up to 16 °C in the Northern Western Mediterranean Sea while, in contrast, Privitera et al. (2011) reported that *A. lixula* of the Ligurian population did not develop below 18 °C as confirmed by our results since *A. lixula* larvae from both adult experimental populations failed completely to develop at 16 °C. Accordingly to Wangensteen et al. (2012), the different thermal responses of development in these two neighbouring populations may be due to genetic differences that affect gametes' performance as well as, possibly, larval ones. On the contrary, *P. lividus* is much more thermosensitive into the thermal window considered optimal for fertilization and development between 16 and 20 °C, with a strongly altered gametogenesis already at 24 °C (Spirlet et al., 2000), especially for populations coming from the Atlantic region. For instance, Garcia Molinos et al. (2016), highlighted that the combined exposure to three levels of pH (8.0, 7.7 and 7.4) and three temperatures (19.0, 20.5 and 22.5 °C) significantly influenced larval growth and survival of an Atlantic population of *P. lividus* with negative effects when lower pH values were combined with the highest temperature. Contrariwise, our results find confirmation from a study conducted by Bressan et al. (1995) where it was observed that the maintenance at 22 °C of *P. lividus* larvae from individuals collected in the North Adriatic Sea resulted in a percentage of survival of around 80 %. Furthermore, our results indicated that the highest tested temperature hide the negative effect of acidification on the calcifying response of *A. lixula*, as also reported in other invertebrates larvae (Byrne et al., 2013).

In the echinoplutei of *Tripneustes gratilla*, negative effects of acidification on larval growth are reduced by moderate warming (about 2 °C above ambient temperature) (Brennand et al., 2010). Another study on the Antarctic sea urchin *Sterechinus neumayeri* showed that increasing temperatures (+1.5 and +3 °C) lead to a 20–28 % increase in OSL at all pH treatments, while OSL was negatively influenced by reduced pH (7.6, 7.8) with a decrease in length about 8–19 % at all the tested temperatures (Ericson et al., 2012), and in a similar study, Brennand et al. (2010) found that an increment of 3 °C improved the larval growth of the sea urchin *T. gratilla* under treatments of different pH, buffering its negative effects. Beside the direct effect that acidification has on calcareous structures, it is possible that both warming and pH impose an energetic cost beyond sustainability with the consequent developmental delay and increased mortality. This hypothesis might find confirm in studies conducted on a longer experimental time where it has been shown that the reduction of spicules of sea urchin larvae can be associated with other negative effects on larval performance. Hart (1991), studying the sea urchin *Dendraster excentricus*, found that the reduction of the post-oral and antero-lateral spicules of the echinoplutei was related to a lower food assimilation efficiency. This result is confirmed by a further study on *D. excentricus* that showed that a pH reduction corresponds to a smaller size of the pluteus stomach along with other negative effects on growth and development (Chan et al., 2011). All this, caused an energy depletion of reserves available for larvae, spending more time in their planktonic phase before reaching the metamorphosis and therefore increasing the risk of predation and mortality (Byrne et al., 2009; Ross et al., 2011). The mortality of larvae as plankton is estimated to exceed 90 % and, in an ocean altered by climate change, this could increase (Thorson, 1950; Gosselin and Qian, 1997). Although smaller larvae survive, they may have reduced competitive capacity at the time of settlement (Hobday and Tegner, 2002; Kurihara et al., 2007; Byrne et al., 2009; Byrne, 2011) and increased post-settlement mortality (Talmage and Gobler, 2009; Dupont et al., 2008; Clark et al., 2009). Clark et al. (2009) investigated the responses on several species of sea urchins, three of which showed a significant reduction in calcification index when larvae were grown under low-pH conditions. The decrease was greatest in the temperate New Zealand species of the genus

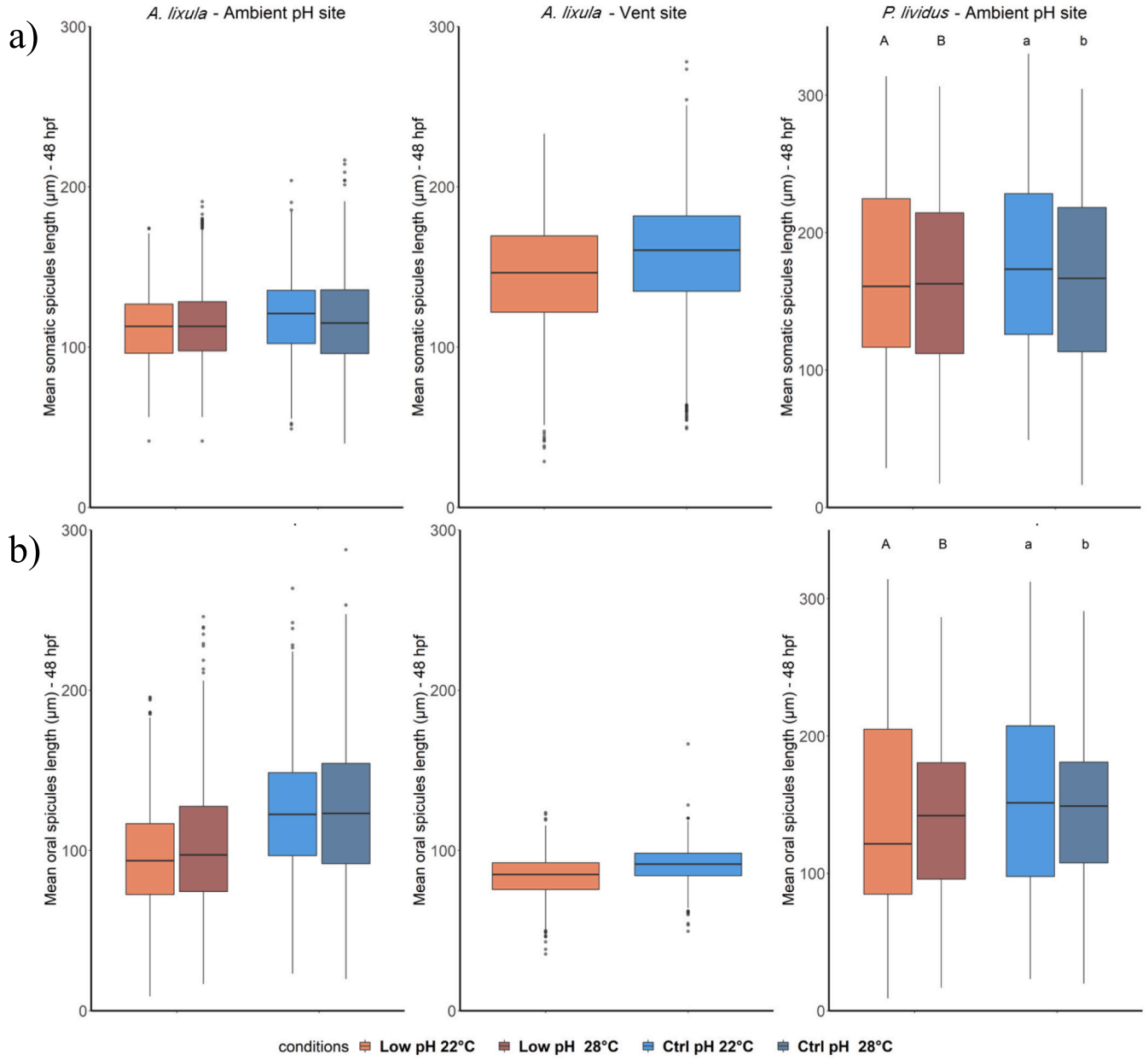

Fig. 6. Mean length ± SD (n = 3) of somatic (a) and oral (b) spicules at 48 h post fertilization. On the X axis the larval exposure conditions are shown. Significant differences ($p < 0.05$) among temperatures at low-pH ($pH_T \sim 7.7$) are represented with capital letters (A-C), while lower-case letters (a–c) were used for control-pH ($pH_T \sim 8.0$). Greek lower-case letters (α–β) represent the significant differences ($p < 0.05$) between pH levels at the same temperature.

Pseudechinus (36.9 %) and *Evechinus* (30.6 %) and was more moderate in the tropical *Tripneustes* (13.8 %).

4.2. Parental buffer effects on larval developmental stages

However, as mentioned above, sensitivity to environmental perturbations may be buffered by maternal anticipatory effects (Marshall and Uller, 2007; Sunday et al., 2011).

Generally, the larger size of larvae or the lower presence of anomalies observed in some families, in relation to both reduced pH and temperature variations, could be due to the transfer of phenotypes more favourable from the mother, which would allow the larvae to allocate more energy in favour of normal somatic growth during development. In both species, experimental families exhibited different responses to both pH and temperature, and their interaction as well, highlighting the fact that within a given population exist a certain level of variation of response to one or more environmental variables.

Furthermore, by comparing the two *A. lixula* populations, our results showed that the effect due to reduced pH was not significant on all growth-related variables considered in larvae from vent site parents, except for larval anomalies and the oral spicules length, when compared to the larvae whose parents lived at ambient-pH of 8.1. In particular, regarding the 24 hpf development frequencies at reduced pH and 22 °C, the percentage of early plutei of the vent site experiment was about 8 % higher than those of the ambient-pH site. At 48 hpf, the percentage of echinoplutei of the vent site experiment reached a difference of 33 % compared to those of the ambient-pH site. As for the spicules growth, although the oral ones of larvae from the vent site parents were generally shorter, the somatic spicules had a length >22 % compared to the larvae of the ambient-pH site experiment, while there was a decrease of 9 % in abnormal larvae.

Since sea urchins spread their gametes in the water column and the

larvae are influenced by marine currents, this difference between *A. lixula* ambient-pH site larvae and the vent site is not to be considered a genetic transgenerational mechanism, but it is more likely due to parent-induced phenotypic changes (*i.e.* acclimatization) in the traits of their offspring without altering the sequence of their DNA (Salinas and Mulch, 2012). It can be defined as a phenotypic change in progeny in response to environmental stress experienced by parents. For example, Jensen et al. (2014) observed that adults of polychaete *Hydroides diramphus* when pre-exposed to different levels of salinity are able to modify the phenotypic plasticity of their gametes, thus influencing the larval performance in response to changes in salinity. Another aspect to be considered in future study is epigenetic inheritance which is gaining considerable ground in literature as a key mechanism in the transgenerational response of marine organisms during exposure to environmental stresses (Vandegehuchte and Janssen, 2014). Stressful environmental conditions trigger beneficial modifications in the gene expression pattern of parents which are transmitted to their offspring, influencing their phenotype. It is believed that these epigenetic changes occur mainly through DNA methylation and the modification of the histone and/or non-coding RNA (Riviere, 2014; Vandegehuchte and Janssen, 2014).

Understanding whether marine invertebrates will have the ability to cope with global environmental change by the end of the century is still a topic of great uncertainty (Donelson et al., 2011; Sunday et al., 2014). Uncertainty depends mostly on the fact that single-generation experiments are limited by the 100-year compression of evolution in a period that is experimentally feasible and fundable (Fitzer et al., 2012) as well as the fact the most studies has been conducted in laboratory where it can be complicated to perform long term exposure that comprehend several generations. For organisms with long generation times (from months to years), such as echinoderms, measuring the response of species across multiple generations is then very difficult (Sunday et al., 2014). Transgenerational responses could facilitate the processes of phenotypic acclimatization between generations in response to the environment, in time scales that may be lower than the expected rate of climate change in the oceans (Donelson et al., 2011). Fitzer et al. (2014) found that in *Mytilus edulis* parental exposure (6 months) and offspring at high levels of CO_2 meant that young individuals did no longer produce aragonite in their shells but only calcite. This variation was justified as a compensatory mechanism, as aragonite was more vulnerable to the under-saturation of calcium carbonate than calcite (Fitzer et al., 2014). Similar results were found for larvae of the adult Antarctic sea urchin, *S. neumayeri*, where parental larvae exposed to a higher temperature and reduced pH for 6 or 17 months had different outcomes (Suckling et al., 2015a, b). The six-month exposure led to a reduction in egg size with a 63 % increase in hatching success compared to parents exposed to current control conditions. The 17-month high exposure has instead led to greater larval success (survival and speed of development).

There is also evidence of a transgenerational response to increased CO_2 from field experiments. Kelly et al. (2013) collected adults of purple sea urchin *S. purpuratus* from two sites characterized by different pH along the north-eastern Pacific coast. Results showed that there was a significant maternal (but not paternal) effect on the size of the larvae that had been raised to high levels of CO_2. Overall, the larval size was reduced in all family lines, but the magnitude of this reduction was significantly lower for the larvae whose mothers were collected from the site with reduced pH.

Regarding the effect of temperature variation on fertilization and early development, there is clear evidence that thermal acclimatization of parents, particularly during oogenesis, can drastically change the thermal tolerance of embryos from sea urchins and other invertebrates (Johnson and Babcock, 1994; Bingham et al., 1997; Rahman et al., 2009). Several generations of tropical and temperate sea urchin species are resistant to temperatures higher than current ones (Byrne, 2011). Therefore, the mother's environmental history can influence the stress tolerance of the offspring. This broad thermotolerance is likely to be transmitted by maternal factors loaded into the eggs during their development in response to environmental temperature and potentially include heat shock protective proteins that influence the superior thermal tolerance of the progeny (Somero, 2002, 2010; Hammond and Hofmann, 2010). Increasing egg reserves by mothers who live in sub-optimal environments is an adaptive strategy used by marine organisms to help offspring survive under non-optimal environmental conditions (Marshall et al., 2008; Sanford and Kelly, 2011; Allan et al., 2014).

5. Conclusion

In this study, the hypothesis of the presence of anticipatory maternal effects on the response of the larvae to environmental stress factors, in both species of sea urchins, was confirmed. Although the population of *P. lividus* of Ischia was found to be much more sensitive to an increase in temperature than of *A. lixula* (thermophilous species), the acclimatization of the latter species to a reduced pH led to a substantial diversification in the larval response to both ocean acidification and ocean warming. Indeed, the progeny of the samples from the vent site, although showing higher resistance to the increase in pCO_2, were completely intolerant to temperature variation. We can also hypothesize that under extreme stress events larvae are not able to compensate for the environmental challenges through an increase in metabolism and maternal effect (Pörtner and Farrell, 2008). Further evidence is needed from the analyses of the energetic content of eggs from the different mothers, since we did not find any particular relationship between the size of the eggs and the responses of the larvae to the selected stressors. To conclude, the approach used in this study can provide valuable information on possible long-term effects and the potential for adaptation of a given population for the forecasted environmental conditions.

CRediT authorship contribution statement

Cristina Palombo: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Antonia Chiarore:** Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Maria Ciscato:** Formal analysis, Investigation, Writing – review & editing. **Davide Asnicar:** Formal analysis, Writing – review & editing. **Alice Mirasole:** Formal analysis, Data curation, Writing – original draft, Writing – review & editing. **Erika Fabbrizzi:** Formal analysis, Data curation, Writing – review & editing. **Nuria Teixidó:** Resources, Writing – review & editing. **Marco Munari:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2023.114700.

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