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ORIGINAL PAPER

Ocean warming modulates the effects of limited food availability on *Paracentrotus lividus* larval development

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Abstract Oceans are becoming warmer due to climate change processes. Marine invertebrates live within a limited range of body temperatures, and functional constraints result at temperature extremes. Furthermore, interactions between temperature and other environmental stressors have the potential to narrow the thermal windows of species. This study assessed the interactive effects of current and predicted conditions of temperature and food availability on the survival, growth, and development of the sea urchin *Paracentrotus lividus*. Samples were collected from the Canary Islands (28°24′N, 16°18′W) in March 2011. Nine two-factor treatments of temperature (19, 20.5 and 22.5 °C) and food level (2000, 1000 and 500 cells mL^{-1}) were tested in laboratory experiments. The temperature and food-level treatments were chosen based on current oceanographic data for seawater off the studied region and from values predicted for the next century in the subtropical eastern Atlantic region. Our results indicated that *P. lividus* larvae survival could be affected by increasing seawater temperatures, in ranges expected to occur over the next century. The negative effects of decreasing food availability on the development of *P. lividus* larvae will be significantly modulated, however, by increasing seawater temperature. These results show that surviving sea urchin larvae are capable of shifting their energy budget to successfully grow and develop under the stressful conditions presented by the combined effects of environmental factors.

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Introduction

Global climate change processes are increasingly impacting the ocean. These changes, triggered by interacting environmental and anthropogenic factors, will affect the ocean in ways that we are only now beginning to understand (Turley et al. 2013). Global warming, a consequence of the emission of gases with greenhouse effects, is one of the most prominent of these climate processes. The impact of global warming on the ocean can have dramatic effects on marine systems. It is thought that seawater temperatures will rise between 2.0 and 4.5 °C by the end of the twenty-first century (IPCC 2013). In addition, ocean warming determines the distribution and adaptability of species, and their survival can be compromised within a specific temperature range (Fields et al. 1993; Lubchenco et al. 1993; Harley et al. 2006; Sunday et al. 2012; Bates et al. 2013, 2014). As a result, the redistribution of species can produce serious ecological problems in specific regions, thereby causing imbalances within local ecosystems.

Oceanographic changes related to global warming will likely affect the trophic structure of ecosystems. Warmer ocean temperatures increase stratification of the surface mixed layer, which hinders the incorporation of nutrients from below that support ocean primary production

(Sarmiento et al. 1998), thereby causing a significant nutrient stress for phytoplankton. Ocean phytoplankton is responsible for approximately half the global biospheric net primary production (Behrenfeld et al. 2001), and thus, long-term changes in ocean primary production can potentially have important consequences for the global carbon cycle.

Temperature is the most important environmental factor controlling the distribution, physiology, morphology, and behavior of marine invertebrates. This factor, more than any other variable, best explains developmental rates in marine invertebrates (Hoegh-Guldberg and Pearse 1995; Byrne et al. 2009). Early developmental stages, such as fertilization, embryogenesis, and morphogenesis, are generally the most sensitive life history phases (Pörtner and Farrell 2008; see Byrne 2011 for review). Temperature can have both positive and negative impacts linked with different physiological processes with potential consequences for fitness (settlement and energy investment in juveniles) (Pörtner and Farrell 2008). Ocean warming improves fertilization (Hagström and Hagström 1959; Mita et al. 1984; Cohen-Rengifo et al. 2013), speeds up larval growth, development, and settlement, and may also impact larval swimming behavior and duration of planktonic life, up to an organism's thermal threshold (Staver and Strathmann 2002; O'Connor et al. 2007; Sheppard-Brennand et al. 2010; see Byrne and Przeslawski 2013 for review). Although a shorter planktonic period reduces exposure to predators, it also has the potential to limit dispersal distance, thereby altering the distribution and genetic connectivity of populations and thus the dynamics of marine populations, broadly considered (López et al. 1998; O'Connor et al. 2007).

Phytoplankton abundance in the ocean is both highly seasonal and spatially heterogeneous and is strongly related to the timing of reproductive events of many species as well as the subsequent survival of feeding larval stages (Platt et al. 2003). During larval development, organisms typically encounter unpredictable feeding environments (Conover 1968). Furthermore, it is thought that food availability will be reduced due to decreasing primary production resulting from climate change processes (Gregg et al. 2003; Turley et al. 2013). For these reasons, the impacts of food availability on the growth and survival of feeding larval forms have been extensively studied (Olson and Olson 1989; Fenaux et al. 1994; Meidel et al. 1999; Vickery and McClintock 2000; Moran and Manahan 2004; Meyer et al. 2007). As feeding larvae grow, changes occur in a suite of correlated larval characters, including physiological rates, larval morphology, and the magnitude of plasticity of feeding structures in response to different food levels (Fenaux et al. 1994; Sewell et al. 2004; McAlister 2007, 2008). Food-limited growth and development could have implications for the life history, ecology, and evolution of species. For example, some organisms have evolved larval feeding strategies that maximize food collection in environments with constantly low conditions of food availability (McAlister 2008). Planktonic larvae have the potential to be food limited, experience longer transport in the seawater, and may be subject to higher rates of mortality directly due to starvation or indirectly due to prolonged periods of exposure to predation (Lamare and Barker 1999). When an organism is stressed to the edges of its ecological niche, the energy required to maintain the necessary physiological mechanisms allowing survival, development, and reproduction increases. Thus, a prolonged exposure to these challenging conditions can lead to unsustainable energetic costs (Dorey et al. 2013). If food limits growth rate, then larvae may often need to clear particles from suspension at higher rates, thereby increasing metabolic costs, and which may account for strikingly different features of larval form and behavior (Fenaux et al. 1994).

Marine organisms are clearly affected directly by changes in specific environmental factors (e.g., temperature, food availability, salinity, etc.) and in an interactive manner among multiple factors. Interactions between two stressors have been shown to be additive, joining the effects of both stressors in isolation (Vinebrooke et al. 2004; Carilli et al. 2009; see Byrne and Przeslawski 2013 for review), or antagonistic, where the combined effect is less than the additive expectation (Carilli et al. 2009; see Byrne and Przeslawski 2013 for review). Organisms are constantly exposed to a range of abiotic and biotic stressors that can be associated, unassociated, or indirectly associated, with global change. Stressors associated with warming are the most direct and pervasive of global change stressors for marine biota, but effects vary among regions, habitats, species, and life history stages (Byrne and Przeslawski 2013).

To examine the interactive effects of temperature and food availability on the development and growth of larvae, we examined the combined effects of food level and temperature using larvae of the sea urchin *Paracentrotus lividus*. *P. lividus* is widely distributed throughout the Mediterranean Sea and the NE Atlantic Ocean from Ireland to the Canary Islands. In this region, winter mixing depth determines the quantity of nutrients available for the spring bloom and can be impacted year to year by variations or trends due to climate change processes (Chavez et al. 2011). In the Canarian Archipelago, the species is found from the lowest intertidal, where it most commonly occupies crevices in tide pools, to around 10-m depth in the subtidal and occasionally down to about 20-m depth (Girard et al. 2012). In this region, the echinoid has the ability to extend its period of sexual maturity (winter and late summer) and exhibits multiple spawning episodes during the year, likely in response to the warmer seawater

temperatures at its southernmost limit of geographical distribution. The planktonic larval stage is estimated to last roughly one month, and settlement occurs in late winter and early spring, when high phytoplankton abundance is found in the water column (Girard et al. 2008).

Materials and methods

Animal collection and spawning

Adult *P. lividus* specimens (test diameter > 24 mm) were collected by scuba divers from subtidal rocky shores between 5- and 10-m depth. Individuals were collected in March of 2011 off the north-east coast of Tenerife Island (Canary Islands; 28°24′N, 16°18′W), during the spawning season of the species (Girard et al. 2012).

Animals were induced to spawn by injection of 2 mL of KCl (0.5 M) through the peristomial membrane. Five males and seven females, randomly selected in order to ensure genetic variability and minimize the probability of fertilization by genetically incompatible gametes (Evans and Marshall 2005), were used, and their respective gametes were mixed prior to fertilization. Sperm was collected dry and kept on ice until usage. Eggs were collected in filtered seawater (FSW). Gametes were combined in a proportion of 1:2400 (egg:sperm) resulting in a high percentage of fertilization (>90 %). Cleaving embryos (two-cell stage) were placed at a density of 15 individuals mL^{-1} in 20 L aquaria filled with FSW and constantly aerated.

Experimental design and seawater chemistry

When the embryos reached the gastrula stage, larvae were distributed into 2-L culture beakers at densities of 2 larvae mL^{-1}. Forty-five culture beakers were maintained in three seawater tables, which served to regulate temperature treatments. Seawater was replaced in each beaker twice a week. At day 3 post-fertilization, larvae were fed with the unicellular red alga, *Rhodomonas lens*. The microalgae strain was provided by 'Spanish Oceanography Institute' and cultured in the laboratory with enriched *F*/2 medium (Guillard and Ryther 1962) at 20 °C and a 24 h/0 h light/dark cycle, with constantly aerated seawater. Algae were separated from the growth medium by centrifugation and then re-suspended in fresh FSW before use.

Larvae of *P. lividus* were raised in nine different replicated two-factor treatments of temperature and food ($n = 5$ for each treatment). Cultures were maintained at a salinity of 36.94 ‰ (±0.38), corresponding to the natural seawater conditions in March at the collection site. Temperature and food availability levels were chosen to cover the present and future natural variability at the sampling region. Therefore, the experiment included three treatments of sea surface temperatures (SST): 19 °C (control: SST in Spring in the Canary Islands), 20.5 °C (present extreme natural variability in Spring and predicted mean SST for the year 2050, IPCC 2007), and 22.5 °C (predicted SST for the year 2100, IPCC 2007). In each treatment of temperature, three different treatments of food availability were carried out: *F*1: 2000 algal cells mL^{-1}, corresponding to current average values in the seawater off the studied region (Baltar et al. 2009); *F*2: 1000 cells mL^{-1}, and *F*3: 500 cells mL^{-1}, in order to simulate future conditions of a reduced primary production as predicted due to climate change effects (Gregg et al. 2003). Although there are limitations of using a single species diet in comparison with a natural diet, a single species diet minimizes variation among food treatments and is a good approach for testing the effects of energy shortage.

To keep constant temperature conditions in the experiments, thermostat coolers and heaters (EHEIM AQUATICS, 50 W) were used. Monitoring of temperature and salinity (WTW handheld conductivity meter COND 315i) was performed daily in 3–5 randomly selected replicate units in each experimental treatment. Experiments were conducted with FSW purified within a recirculating system provided with DRYDEN AQUA active filter media (AFM) bio-crystals; 50-μm and 10-μm UNICEL polyamide paper filters; and a UV-C AQUAEL 11 W filter. The seawater was prepared with the proper temperature for each treatment before usage, and the appropriate amount of food for each treatment was added every day. To maintain food levels, cell concentrations were calculated and the appropriate quantity of algae was then added to each beaker to reach the target value for each treatment.

Biological measurements

Larvae were sampled for a period of approximately one month (29 days) in order to quantify survival, growth, and larval development. Survival was estimated as the number of living larvae in each combined treatment at the end of the experiment. To account for larval growth and development, several larvae in each replicate beaker for each combination treatment of temperature and food were photographed every other day ($n = 15$ per treatment and experimental day) using a digital camera mounted on a trinocular microscope. Morphological parameters were measured on each larva, starting on day 3 post-fertilization: body length (BL), post-oral arm length (PL), and two perpendicularly oriented stomach diameters (S1 and S2) (Fig. 1). Stomach volume (SV) was then calculated as $SV = 4/3\ \pi\ ((S1 + S2)/4)^3$ (Dorey et al. 2013).

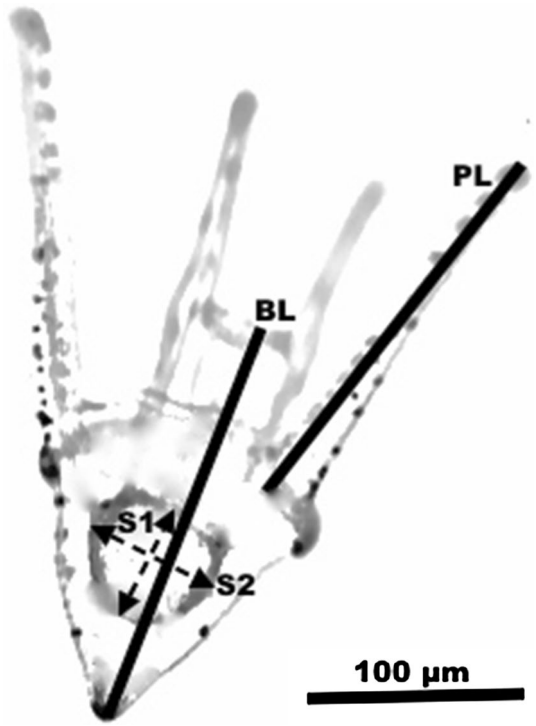

Fig. 1 Morphometric measurements taken for each *Paracentrotus lividus* larvae: body length (BL), post-oral arm length (PL), and stomach diameters (*S*1 and *S*2)

Data analyses

In order to assess the interactive effects of seawater temperature and food level on larval survival, data were analyzed by means of a two-way permutational analysis of variance (PERANOVA) (Anderson 2001) with temperature (3 levels) and food (3 levels) as fixed factors.

To evaluate the interactive effects of temperature and food level on larval body length (BL) and on larval post-oral arm length (PL), three-way permutational analyses of variance (PERANOVAs) were performed. A three-way design was carried out with temperature (3 levels), food (3 levels), and time (7 levels) used as fixed factors. The same design, but with 6 levels of time, was used to analyze the interactive effects of temperature and food availability on stomach volume (SV) (PERANOVA).

Table 2 Results of the two-way permutational ANOVA analyzing larval survival of *Paracentrotus lividus*

Source	*df*	SS	MS	Pseudo-F	*p* (perm)
T	2	64.13	32.07	3.49	0.023
Food	2	58.13	29.07	3.16	0.034
T × Food	4	124.13	31.03	3.38	0.011

The factors included in the model are: *T* Temperature and Food availability

In all analyses of variance, we used Euclidean distances of raw data and 4999 permutations of the appropriate exchangeable units (Anderson 2004). Significant terms in the full models 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. All statistical analyses were carried out using PRIMER 6 & PERMANOVA+ v. 1.0.1 software.

Results

Physicochemical parameters of seawater during the larval experiments are given in Table 1. Target temperature levels were achieved in each replicated treatment (18.91 ± 0.29; 20.54 ± 0.28; 22.33 ± 0.30 °C). A significant interaction of factors 'Temperature × Food' was found when analyzing larval survival (Table 2), meaning that the larval response to food level depended on temperature treatment. An a posteriori pairwise test revealed that at 19 °C, there was significantly higher survival at the control food treatment of 2000 cells mL^{-1} (*F*1) than at the decreased levels of food availability. At 22.5 °C, only increased survival at food level *F*1 compared with *F*2 (1000 cells mL^{-1}) was detected. No significant differences were detected at a seawater temperature of 20.5 °C (Table 3; Fig. 2). Comparison within temperatures revealed significantly higher larval survival at 19 °C, albeit only at control food conditions of 2000 cells mL^{-1} (*F*1). No significant differences in survival were found in the other reduced food treatments *F*2 and *F*3 (500 cells mL^{-1}) (Table 3; Fig. 2).

Table 1 Physicochemical seawater parameters for each experimental treatment tested (*F*1: 2000 cells mL^{-1}; *F*2: 1000 cells mL^{-1}; *F*3: 500 cells mL^{-1})

	19 °C			20.5 °C			22.5 °C		
	*F*1	*F*2	*F*3	*F*1	*F*2	*F*3	*F*1	*F*2	*F*3
$T_{(n=28)}$	18.99 ± 0.30	18.97 ± 0.38	18.80 ± 0.11	20.25 ± 0.29	20.74 ± 0.15	20.61 ± 0.13	22.19 ± 0.13	22.38 ± 0.31	22.43 ± 0.38
$S_{(n=28)}$	36.67 ± 0.40	37.24 ± 0.41	37.02 ± 0.45	36.82 ± 0.38	36.93 ± 0.24	36.86 ± 0.35	37.00 ± 0.33	36.90 ± 0.38	37.01 ± 0.29

T seawater temperature (mean ± SD); *S* salinity (mean ± SD)

Table 3 Results of pairwise tests examining the significant interaction of factors 'Temperature × Food' obtained in the permutational ANOVA on larval survival of the sea urchin *Paracentrotus lividus* in laboratory experiments

Comparisons	19 °C		20.5 °C		22.5 °C	
	t	*p* (perm)	*t*	*p* (perm)	*T*	*p* (perm)
*F*1 versus *F*2	1.87	0.062	0.14072	0.875	2.45	0.037
*F*1 versus *F*3	2.41	0.009	0.75593	0.460	0.62	0.534
*F*2 versus *F*3	1.23	0.257	0.90536	0.415	1.00	0.337
Comparisons	*F*1		*F*2		*F*3	
	t	*p* (perm)	*t*	*p* (perm)	*T*	*p* (perm)
19 versus 20.5	2.01	0.056	0.13	0.896	0.43	0.675
19 versus 22.5	2.34	0.011	1.62	0.149	0.74	0.477
20.5 versus 22.5	0.79	0.439	1.55	0.150	0.61	0.585

Combined effects of temperature (19, 20.5, and 22.5 °C) for pairs of levels of factor Food (*F*1: 2000 cells mL^{-1}; *F*2: 1000 cells mL^{-1}; *F*3: 500 cells mL^{-1}) and effects of Food treatments for pairs of levels of factor temperature are shown

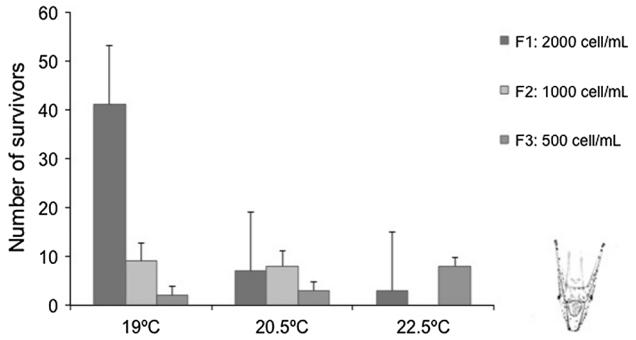

Fig. 2 Number of survivors (mean ± SD) in each combined treatment of temperature (19, 20.5, and 22.5 °C) and food availability conditions (*F*1: 2000 cells mL^{-1}; *F*2: 1000 cells mL^{-1}; *F*3: 500 cells mL^{-1}) at the end of the combined experiment

Results of the PERANOVAs analyzing the body length and post-oral arm length measurements showed, in both cases, a significant three-way interaction of factors 'Temperature × Food × Time' (Table 4a, b). These results indicate that the effects of temperature and food availability on body and post-oral arm length varied significantly across time during the larval period. Pairwise tests showed a significant trend toward shorter BL (Online Resource 1A, Table 5; Fig. 3) and PL (Online Resource 2A, Table 5; Fig. 4) with decreasing food availability at 19 °C, particularly during the last days of larval development. On the contrary, a significant trend toward larger morphometric measurements was detected with increasing temperature at each food treatment (Online Resource 1B, Online Resource 2B, Table 5; Figs. 3, 4).

The analysis of SV showed a significant interaction of factors 'Temperature × Food × Time' (Table 4c). These results indicate that the influence of temperature and food availability on stomach volume varied significantly across time during the larval period. Pairwise analyses detected a trend toward larger SV with decreasing food at 20.5 and 22.5 °C, but not at 19 °C where no consistent differences

Table 4 Results of the three-way permutational ANOVA testing larvae development analyzing (a) body length, (b) post-oral arm length, and of (c) the three-way PERANOVA assessing stomach volume (SV) of the sea urchin *Paracentrotus lividus*

Source	*df*	SS	MS	Pseudo-F	*p* (perm)
(*a*) *Body length*					
T	2	0.92	0.46	79.56	0.001
Food	2	0.26	0.13	22.63	0.001
Ti	6	6.27	1.04	18.54	0.001
T × Food	4	8.20E−2	2.05E−2	3.56	0.008
T × Ti	12	0.15	1.25E−2	2.17	0.014
Food × Ti	12	0.17	1.39E−2	2.41	0.005
T × Food × Ti	24	0.26	1.07E−2	1.85	0.007
(*b*) *Post-oral arm length*					
T	2	0.85	0.42	44.59	0.001
Food	2	0.26	0.13	13.73	0.001
Ti	6	4.42	0.74	77.56	0.001
T × Food	4	0.24	5.97E−2	6.28	0.002
T × Ti	12	0.35	2.91E−2	3.06	0.001
Food × Ti	12	0.21	1.72E−2	1.81	0.038
T × Food × Ti	24	0.70	2.90E−2	3.06	0.001
(c) Stomach volume					
T	2	4.32	2.16	89.58	0.001
Food	2	0.17	8.45E−2	3.50	0.014
Ti	5	0.95	0.19	7.92	0.001
T × Food	4	0.18	4.60E−2	1.91	0.070
T × Ti	10	1.73	0.17	7.16	0.001
Food × Ti	10	0.54	5.38E−2	2.23	0.003
T × Food × Ti	20	0.87	4.35E−2	1.80	0.004

Factors included are: *T* Temperature, Food availability, *Ti* Time

Table 5 Mean values of body length (BL, mm), post-oral arm length (PL, mm), and stomach volume (SV, mm^3) (mean ± SD) of *Paracentrotus lividus* larvae for each combined treatment of temperature (19, 20.5, and 22.5 °C) and food availability conditions (*F*1: 2000 cells mL^{-1}; *F*2: 1000 cells mL^{-1}; *F*3: 500 cells mL^{-1})

	19 °C			20.5 °C			22.5 °C		
	*F*1	*F*2	*F*3	*F*1	*F*2	*F*3	*F*1	*F*2	*F*3
BL	0.392 ± 0.14	0.367 ± 0.13	0.318 ± 0.09	0.449 ± 0.12	0.415 ± 0.11	0.407 ± 0.12	0.470 ± 0.12	0.441 ± 0.13	0.439 ± 0.10
PL	0.335 ± 0.13	0.302 ± 0.10	0.257 ± 0.08	0.400 ± 0.12	0.357 ± 0.13	0.366 ± 0.13	0.391 ± 0.14	0.356 ± 0.13	0.388 ± 0.13
SV	0.008 ± 0.010	0.006 ± 0.004	0.006 ± 0.006	0.012 ± 0.008	0.015 ± 0.014	0.018 ± 0.016	0.027 ± 0.028	0.038 ± 0.035	0.038 ± 0.045

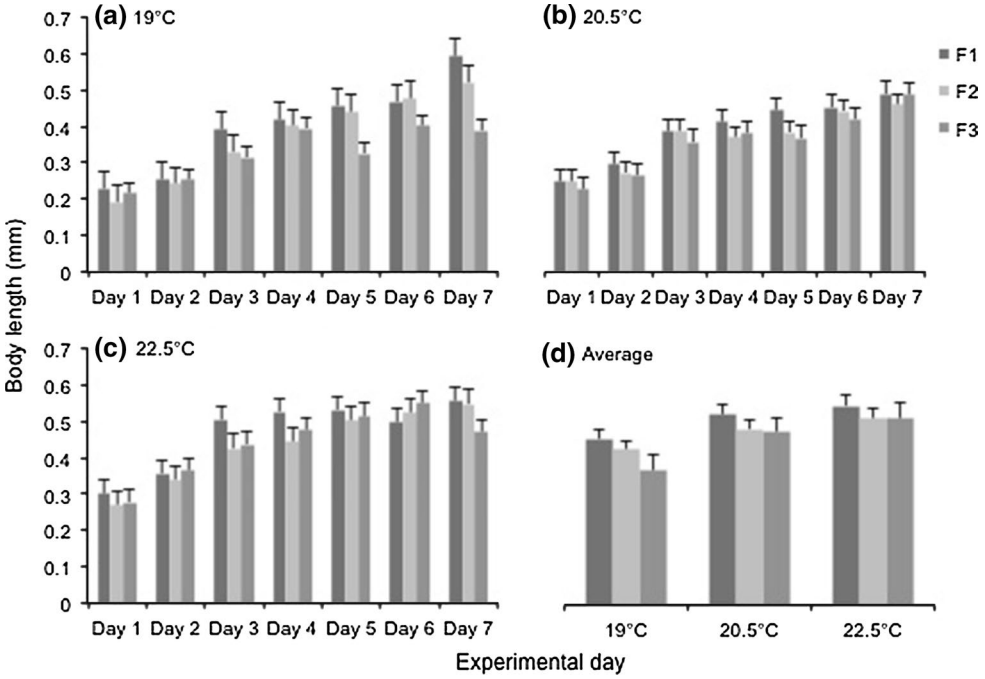

Fig. 3 Body length (mm) of larvae (mean ± SD) of the sea urchin *Paracentrotus lividus* at combined experiments testing the effects of seawater temperature (**a** 19 °C; **b** 20.5 °C; **c** 22.5 °C) and food availability conditions (*F*1: 2000 cells mL^{-1}; *F*2: 1000 cells mL^{-1}; *F*3: 500 cells mL^{-1}). **d** Overall mean values for the experiment are given. *x*-axis shows sampling days, with experimental day 1 corresponding to day 4 post-fertilization and sampling performed in subsequent experimental days every 48 h

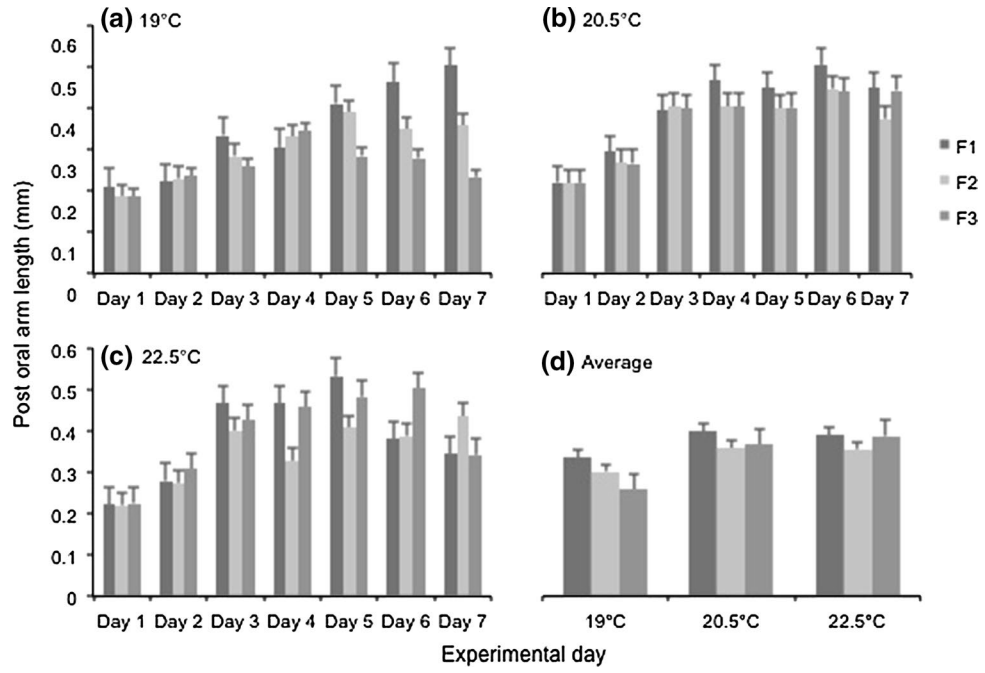

Fig. 4 Post-oral arm length (mm) of larvae (mean ± SD) of the sea urchin *Paracentrotus lividus* at combined experiment testing the combined effects of seawater temperature (**a** 19 °C; **b** 20.5 °C; **c** 22.5 °C) and food availability conditions (*F*1: 2000 cells mL^{-1}; *F*2: 1000 cells mL^{-1}; *F*3: 500 cells mL^{-1}). **d** Overall mean values for the experiment are given. *x*-axis shows sampling days, with experimental day 1 corresponding to day 4 post-fertilization and sampling performed in subsequent experimental days every 48 h

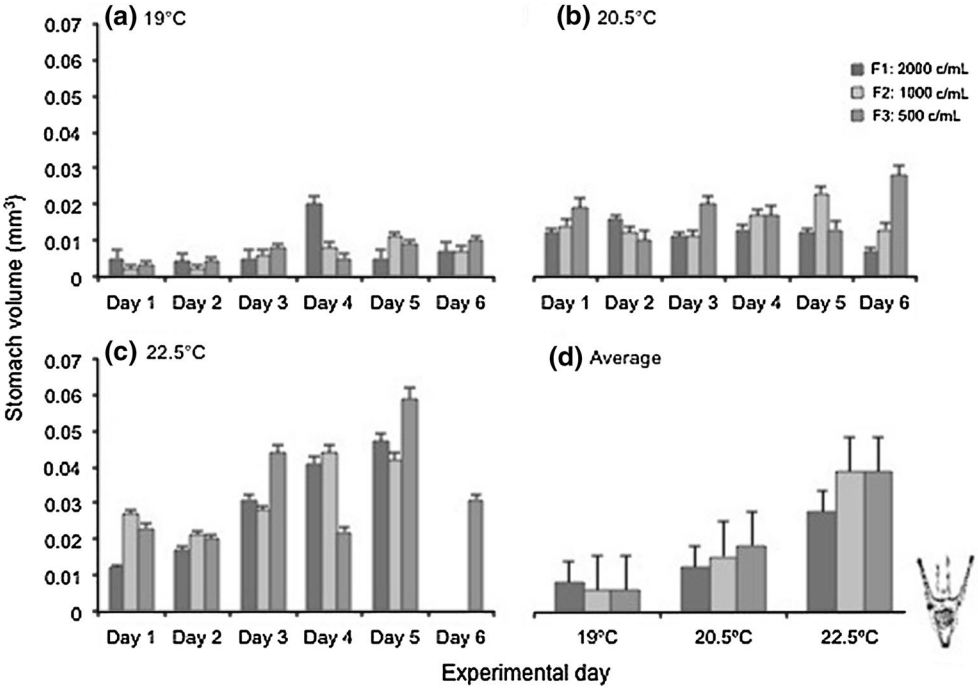

Fig. 5 Stomach volume (mm^3) of larvae (mean ± SD) of the sea urchin *Paracentrotus lividus* at combined experiment testing the combined effects of seawater temperature (**a** 19 °C; **b** 20.5 °C; **c** 22.5 °C) and food availability conditions (*F*1: 2000 cells mL^{-1}; *F*2: 1000 cells mL^{-1}; *F*3: 500 cells mL^{-1}). **d** Overall mean values for the experiment are given. *x*-axis shows sampling days, with experimental day 1 corresponding to day 6 post-fertilization and sampling performed in subsequent experimental days every 48 h

between food treatments were found (Online Resource 3A, Table 5; Fig. 5). Also, a significant trend toward greater SV with increasing temperatures at each treatment of food was detected (Online Resource 3B, Table 5; Fig. 5).

Discussion

Our results suggest that *P. lividus* larvae are widely tolerant to current levels of environmental variation in seawater temperature and food availability. However, *P. lividus* larvae appear to have greater sensitivity (detrimental effects on larval development) to near-future predicted levels for both of these parameters. The combined effects of the climate change-related environmental factors we examined resulted in a reduction in fitness at the warmer region of the species thermal range, thereby affecting larval survival and development. These detrimental effects may have striking consequences for the future performance of a key herbivore species (Pörtner and Farrell 2008) and thus for the stability of marine ecosystems.

Larval survival was highest at the species' optimal temperature (19 °C) and within the highest food supply (2000 cells mL^{-1}). Larval survival was reduced at the optimal raising temperature with lower levels of food. Fewer survivors were detected in seawater conditions representative of warming (20.5 and 22.5 °C), with respect to the control temperatures, with nonsignificant effects of the food availability treatments in these extreme conditions. More powerful survival analyses would be desirable to ensure a real effect on survival, however. Alternatively, other studies have reported robustness (i.e., survival) of *P. lividus* larvae against environmental factors such as ocean warming and acidification, up to critical thresholds (Martin et al. 2011; Privitera et al. 2011; García et al. in review). Responses to environmental changes and the capacity for adaptation will differ among populations and species (Kelly et al. 2013). *P. lividus* has a wide latitudinal range, can be found in intertidal to subtidal environments, and has the capacity to cope with high environmental variability (Moulin et al. 2011). Therefore, the species possesses strategies for inhabiting coastal areas where stress and disturbance are frequent and its thermal tolerance window is broad, suggesting considerable plasticity for a number of different phenotypes (Catarino et al. 2012).

The fact that higher temperatures facilitate invertebrate larval growth and development up to a thermotolerance threshold is well known in the literature (Hoegh-Guldberg and Pearse 1995; see Byrne 2011 for review). Recent studies show that there are important additive to synergistic effects of food concentration and elevated sea surface temperature on larval development (Uthicke et al. 2015). We found a significant trend toward larger sizes for each of the morphometric variables (BL, PL and SV), with increasing seawater temperature at each treatment of food availability. Changes in food availability are known to result in shifts in allocation and timing between ephemeral larval structures (paired arms) and structures that are retained in the post-metamorphic juvenile (echinus rudiment and stomach) (Strathmann et al. 1992). Within this study, we observed

a significant trend toward shorter larval BL and PL with decreasing food availability at the optimal temperature for the species (19 °C).

Our results suggest that warmer ocean temperatures, as predicted for future climate change scenarios, may compensate for lower food availability. Thus, the positive effects that result from more rapid development may modulate the negative effects of low food, in part. Similar response trade-offs between increasing ocean warming and other environmental factors, i.e., ocean acidification, have been reported for other echinoid species (Sheppard-Brennand et al. 2010; Byrne et al. 2013). Likewise, in a previous study with *P. lividus,* we found that a slight ocean warming (20.5 °C) mitigated the negative effects of ocean acidification on larval growth and development, but enhanced the sensitivity at more extreme high-temperature regimes (22.5°) (García et al. in review). We did not observe the same pattern with food availability conditions in the present study, however.

Interactive stressors may have the potential to narrow the thermal windows of species (Pörtner and Farrell 2008). Our results show for this species, however, that the food levels we tested do not have the potential to narrow its thermal window closer to its thermal threshold. In theory, if baseline metabolism is far from its optimal value, the organism is not energy limited and an increase in metabolism can lead to a positive response. In contrast, if baseline metabolism is closer to its optimal value, any increase in metabolism will lead to a negative response, and under extreme chronic metabolic stress, the effect could even be lethal (Pörtner and Farrell 2008). Our results suggest that in the most extreme conditions of seawater temperature and food availability we tested (22.5 °C/500 cells mL^{-1}), *P. lividus* larvae are far from their critical thermal and food thresholds and their thermal window is likely wider than the window for other environmental factors such as pH, as in the case of ocean acidification (García et al. in review).

Some studies have shown that larvae with a consistent lack of food supply develop longer arms to increase the possibility of collecting food particles (Fenaux et al. 1994; McAlister 2008). The shift in allocation of resources from the stomach and echinus rudiment to the arms and ciliated band when food is scarce could therefore increase larval capacity to successfully catch food and increase growth rate (Strathmann et al. 1992; Miner 2005). In contrast to these findings, we did not detect evidence for an increase in size of post-oral arm length of larvae exposed to food shortage. Plasticity of arm length may be an evolutionary strategy that results in greater food gathering capability for larvae inhabiting temperate habitats (McAlister 2008, Soars et al. 2009). In fact, this pattern in pluteus larvae has been demonstrated primarily in temperate cold-water species (Boidron-Metairon 1988; Hart and Scheibling 1988; Sewell et al. 2004). Usually, this trend is not observed in ecosystems with a high variability of environmental factors, more typical in mean latitudes. Although there are certainly exceptions to the observation (e.g., Fenaux et al. 1994), the general pattern suggests that there may be a latitudinal gradient in phenotypic plasticity of larval feeding structures (McAlister 2008). On the contrary, larvae living in environments with constantly low food availability conditions, which is the case of the oligotrophic waters off the Canary Islands, may express a constant long arm length phenotype likely increasing the food gathering capability of a given larva, as has been reported by Caribbean species of the genera *Diadema* (McAlister 2008).

We hypothesize that in conditions of scarce food availability and with rising seawater temperatures, larvae could shift allocation of energetic reserves toward increasing stomach volume in order to maximize food digestion capacity and maintain its rate of growth (Strathmann et al. 1992; Miner 2005). In support of this hypothesis, we detected a trend toward larger SV with the gradual shortage of food at 20.5 and 22.5 °C, although this pattern was not consistent at 19 °C. This result could be a consequence of a shift in the energy budget (uncompensated increased energy costs) as has been hypothesized by Stumpp et al. (2011).

In conclusion, the survival of *P. lividus* larvae could be affected by increasing seawater temperatures, in ranges expected to occur over the next century. However, our results suggest that surviving sea urchin larvae may be capable of shifting their energy budget to successfully develop and grow under the stressful conditions presented by the combined effects of environmental factors. Our results indicate that increasing temperatures modulate the negative effects of decreasing food availability on *P. lividus* larval development. While our study sheds light on the interactive effects of environmental stressors, experiments assessing novel and untested multiple stressors are needed to further evaluate organismal response. Understanding how multiple stressors interactively control thermal windows will provide useful information for making predictions about the adaptability of species to future climate change conditions.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard Experiments comply with the current Spanish laws.

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