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Morphological response of the larvae of *Arbacia lixula* to near-future ocean warming and acidification

Giulia Visconti¹, Fabrizio Gianguzza², Emanuela Butera¹, Valentina Costa¹, Salvatrice Vizzini¹, Maria Byrne³ and Paola Gianguzza^{1*}

¹Dipartimento di Scienze della Terra e del Mare (DiSTeM), Università di Palermo, CoNISMa, Via Archirafi 22, Palermo I-90123, Italy

²Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche (STeBiCeF), Università di Palermo, Via Archirafi 22, Viale delle Scienze, Ed. 16, Palermo 90100, Italy

³Coastal and Marine Ecosystems Group and Schools of Medical and Biological Sciences, University of Sydney, Sydney, NSW, Australia

*Corresponding author: tel: +39 (0) 9123862857; fax: +39 (0) 9123891847; e-mail: paola.gianguzza@unipa.it.

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The distribution of the sea urchin *Arbacia lixula*, a warm affinity species, has been expanding in the Mediterranean Sea. To address questions on potential for future success of this species in the region, the thermotolerance of larval development was investigated in context of regional warming. The larvae were reared in present day spawning period (20 °C) and warming conditions (+4 = 24 and +6 = 26 °C). As the calcifying larvae of sea urchins are vulnerable to stunted growth caused by ocean acidification, the impact of lower pH (–0.3 pH units) on larval development was also investigated in combination with warming. Morphological traits of the larvae, post-oral length arms, overall length of larvae and body length, were affected by increased temperature across pH treatments, indicating that for the larvae of southern Mediterranean population here, 24 °C appears to approximate the optimal temperature for development. A slightly negative effect of pH was evident. Increased temperature ameliorated the stunting effect of acidification on growth. The thermal tolerance of *A. lixula* development overlaps with projections for warming in the region by 2100 and also indicates that this species has acclimatized or adapted its reproductive biology to the broad environmental conditions of the Mediterranean Sea. Due to the broad thermal range (~10 °C) of development of *A. lixula* across its distribution, this species is likely to be a winner in the climate change stakes. The broad thermal tolerance of the larvae is likely to assure population connectivity between Mediterranean sub-basins populations. The continued success of *A. lixula* can have a strong consequences for the ecological structure of Mediterranean rocky habitat.

Keywords: *Arbacia lixula*, climate change, larvae morphology, sea urchin, thermal history.

Introduction

As the ocean warms and the rate of climate change increases, marine ecosystems are changing in unpredictable ways with many species moving towards polar latitudes following their thermal niche, while others remain (Burrows *et al.*, 2011; Sunday *et al.*, 2015; Byrne *et al.*, 2016; Poloczanska *et al.*, 2016). This change in species distributions is creating novel communities and species interactions (Burrows *et al.*, 2011; Harley, 2011; Sunday *et al.*, 2015). Changes are particularly important when the new interactions involve ecosystems engineers such as herbivorous sea urchins and

fishes (Ling *et al.*, 2008; Gianguzza *et al.*, 2011; Kroeker *et al.*, 2013; Milazzo *et al.*, 2013; Wangenstein *et al.*, 2013; García *et al.*, 2016). For regions where marine heat waves are an issue, like in the Mediterranean Sea, it is important to understand the thermal biology of key species and to predict which species are sensitive to changing climate (Hart and Scheibling 1988; Coma *et al.*, 2009; Garrabou *et al.*, 2009; Marbà and Duarte 2010; Harley 2011; Wernberg *et al.*, 2013; Lacoue-Labarthe *et al.*, 2016).

This scenario makes the Mediterranean Sea a model system to investigate the relationship between species thermotolerance and

their ability to survive and recover from warming and to increase their population and expand their range. In the North Western Mediterranean (NWM), one of the coldest areas in the Mediterranean, many range expansions of warm affinity species have been recorded (Bianchi, 2007; CIESM 2008; Lejeune *et al.*, 2010; Gianguzza *et al.*, 2011; Parravicini *et al.*, 2015). Among the most high profile of these are the thermophilous southern Mediterranean species that are responding to warming of the NWM, including the ornate wrasse *Thalassoma pavo* (Lowe, 1843), the yellowmouth barracuda *Sphyræna viridensis* (Cuvier, 1829), the dolphinfish *Coryphaena hippurus* (Linnaeus, 1758), the crustacean *Heminyxis margalefi* (Alcaraz, Riera & Gili, 1986) and the orange coral *Astroides calycularis* (Pallas, 1766). Other warm thermophilous species such as the black sea urchin *Arbacia lixula* (Linnaeus, 1758) are also increasing their population density and distribution in northern areas (Francour *et al.*, 1994; Wangensteen *et al.*, 2013).

Arbacia lixula is one of the most abundant echinoids in shallow rocky habitats of the southern Mediterranean and occurs along the Atlantic coast of Spain, Portugal and North-western Africa and in the Western Atlantic, off the coast of Brazil (Guidetti and Dulcic, 2007; Gianguzza and Bonaviri 2013). *A. lixula* has considerable trophic plasticity, ranging from omnivory to strict carnivory (Wangensteen *et al.*, 2011; Agnetta *et al.*, 2013). The scraping predatory behaviour of this species can play a dominant role in driving the switch between one complex state, dominated by a stratified assemblage of several erect macroalgae, to a simpler one dominated by encrusting algae the so-called 'barren ground' (Bonaviri *et al.*, 2011; Wangensteen *et al.*, 2012; Agnetta *et al.*, 2013). *A. lixula* has a tropical affinity (Stefanini, 1911; Mortensen, 1935; Tortonese, 1965) and probably spread through the Mediterranean in the Upper Pleistocene (Wangensteen *et al.*, 2012). Phylogeographic genetic evidence indicates that this species is a recent thermophilous (warm tolerant *sensu* Kempf 1962; Francour *et al.*, 1994; Fenaux, 1968) colonizer of the Mediterranean (Wangensteen *et al.*, 2013).

Increasing evidence suggests that on-going warming of the Western Mediterranean is creating an environment increasingly favourable for the reproduction and development of *A. lixula* (Francour *et al.*, 1994; Guidetti and Dulcic, 2007; Gianguzza *et al.*, 2011; Privitera *et al.*, 2011; Lessios *et al.*, 2012; Wangensteen *et al.*, 2012). Considering the great colonizing potential shown by *A. lixula*, including its ability to cross trans-oceanic barriers to gene flow (Wangensteen *et al.*, 2012), and its ecological importance in coastal ecosystems to maintain barren habitats (Bonaviri *et al.*, 2011), it is critical to evaluate if the success of this species may be modulated by near future ocean warming and acidification. A single stressor study of *A. lixula* from the NWM involving three temperatures at control pH showed that the larvae generated from parents acclimated to $\sim 20^{\circ}\text{C}$ (September, 2012, <http://www.meteoestartit.cat>) are tolerant to cooler conditions ($\Delta -6^{\circ}\text{C}$) but larval growth and survival was highest at 19°C (Wangensteen *et al.*, 2013). At 19°C and pH_T 7.7 larvae were smaller but had a high survival rate to metamorphosis and the early juvenile (Wangensteen *et al.*, 2013). Interestingly, although the larvae were smaller, development was not delayed as those reared at low pH metamorphosed at the same time as controls, albeit producing smaller juveniles (Wangensteen *et al.*, 2013).

Here we investigated the impact of warming on larval development of *A. lixula* from the warm part of its range in the

Mediterranean providing data to compare with the response of the development of this species in its cooler range (see Wangensteen *et al.*, 2013). Our previous study showed that the fertilization success of *A. lixula*, from this region is robust to acidification ($\Delta\text{pH} -0.3$ units) and warming ($+6^{\circ}\text{C}$) (Gianguzza *et al.*, 2014). The temperature treatments used included near-future projections for climatic warming and occasional temperature spikes in the southwestern Mediterranean (Intergovernmental Panel on Climate Change *et al.*, 2013). We determined if warming ameliorated the negative effects of acidification on larval growth, as reported for the echinoplutei of other sea urchins (Sheppard Brennand *et al.*, 2010; Byrne *et al.*, 2013a). Environmental stressors including ocean acidification can alter the body profile of echinoplutei causing marked left–right asymmetry in arm length and changing the allometric relationship between body size and arm length (Kurihara and Shirayama, 2004; Sheppard Brennand *et al.*, 2010; Byrne *et al.*, 2013b). Change in these traits can affect swimming and feeding performance (Hart and Strathmann, 1994; Soars *et al.*, 2009; Chan *et al.*, 2011). Thus, we also investigated the impact of warming and acidification on left–right asymmetry and body allometry as key functional traits. We tested two hypotheses with respect to larval morphology: (i) that both stressors would exert a significant influence on larval body profile; including larval size and shape and (ii) that increased temperature would reduce the negative effect of decreased pH on larval growth.

Material and methods

Thermal condition, specimens collection and spawning of population

The sea surface temperatures (SST) (2004–2008) in the area of the Southern Tyrrhenian Sea (Western Mediterranean Sea) where the *A. lixula* were collected has an annual range from 15 to 27°C (5–7-m depth) (laboratory data). *A. lixula* in the studied area has an extended reproductive cycle, with a spawning from January to September (G. Visconti *et al.*, unpublished data), thus the developing gametes and larvae can experience a large temperature range. However, peak spawning occurs in May, when SSTs are around 20°C . Our investigation coincided with the peak spawning period to ensure high-quality gametes.

Arbacia lixula specimens (test diameter without spines 30–35 mm) were collected by SCUBA diving in May 2011 from the upper infralittoral (3–6 m) of “Capo Gallo-Isola delle Femmine MPA” (Palermo) in the Southern Tyrrhenian Sea ($38^{\circ}11'45''\text{N}$ – $013^{\circ}14'58''\text{E}$) and transported in the laboratory. The specimens were kept in large flow through aquaria for 1 day under natural condition (pH 8.0 units and temperature 20°C) before the experiment.

Fertilization and culture methods

Gametes from multiple males and females were used to establish independent populations of embryos. This approach was taken to mimic the population spawning scenario and to reduce potential artefact due to male–female incompatibility (Evans and Marshall, 2005; Byrne, 2012; Foo *et al.*, 2012; Schlegel *et al.*, 2012). Gonads from three randomly selected females and males were gently removed and rinsed in filtered seawater from sampling area (FSW, Millipore filter $0.45\ \mu\text{m}$). The eggs (ca. $1.5\ \text{ml}^{-1}$) were placed in 1000 ml of experimental FSW for 15 min prior to fertilization. The sperm, collected and stored in a Petri dish on ice were activated,

mixed (3000 sperm/ml) and added to the eggs (200 μ l/l). Six independent populations of embryos were reared in experimental conditions to the 48 h unfed 4-armed larvae stage (3 temp \times 2 pH \times 6 populations = 12 jars). Fertilization as indicated by the presence of cleaving embryos or a fertilization envelope was checked microscopically after 4 h ($n = 50$ embryos per treatments), with a 60–80% success.

Experimental treatments

In the previous study, 27°C was identified as lethal temperature for fertilization success of *A. lixula* (Gianguzza et al., 2014). Over its long spawning period in South Mediterranean *A. lixula* experiences a wide temperature range (20–27°C) with peak spawning at 20°C. The control temperature used in the experiment (20°C) reflected this peak and the recent thermal history of *A. lixula* adults prior to collection (May). It was expected that higher temperatures (as 24 and 26°C) would accelerate development and increase malformation of embryos (Bhaud et al., 1995; Reitzel et al., 2004; Somero, 2010). The maximum temperature we used was 26°C, a temperature potentially encountered by larvae generated during the major spawning peak. As larvae generated in May are most likely to experience temperatures of 22–24°C (in June–July) over their 2-month planktonic period we used 24°C as medium temperature for the experiment. Thus three temperatures were used (control=20, +4 = 24, +6 = 26°C) combined with two pH_{NBS} (control=pH 8.2, experimental=pH 7.9) levels as expected over coming decades and beyond (for temperature). The pH was adjusted by bubbling CO₂ until the target was reached, while temperature was maintained using an immersion heater (Tetra HT25, Spectrum Brands Italia S.r.l.). Cultures were maintained in constant temperature room and stirred gently using a motor driven paddle for 48 h. Each treatment included a jar control without larvae to monitor experimental conditions. During the experiment, temperature and pH_{NBS} of each jar was monitored five times in 48 h (every 9 h). The pH was measured by pH meter (Crimson GLP21) calibrated with NBS buffers. The carbonate system parameters (p_{CO_2} , Ω_{Ca} , and Ω_{Ar}) were calculated using pH_{NBS}, temperature, salinity (38) and alkalinity determined by potentiometric titration (A_T 2.500 μ mol/kg) using CO2SYS (Lewis and Wallace, 1998) with dissociation constants from the study by Mehrbach et al. (1973) refitted by Dickson and Millero (1987) (Table 1).

Larval collection, photography, and measurements

Three 1-ml samples were collected from each experimental culture at 48 h and fixed with few drops of formaldehyde (10% in FSW). The first 75 larvae randomly sampled for each treatment

were photographed for morphological measurement using a digital camera mounted on a compound microscope (Olympus BX50). Morphological measurements were documented, using ImageJ software (NIH, USA). Three morphological traits of larvae were measured: post-oral length (POL) arms, overall length of larvae (OL), and body length (BL) (Figure 1). The mean length of the two POL arms was determined and the absolute difference in their length was calculated as a measure of asymmetry (ASY). Lastly, the body profile (SHAPE) indicated by the OL (mean of right and left arms): BL ratio was calculated.

Statistical analysis

For each of three morphological measures (POL, OL, and BL), 25 larvae were randomly selected from the photographic pool. Thus, only one of these measures was taken into account per larva. This design was done to avoid interdependence among morphological measures within individual larvae. For each morphological variable (POL, OL, and BL) data were analysed by 2-factor ANOVA with temperature and pH as fixed factors. To evaluate ASY and SHAPE, data for all 75 larvae were analysed using a 2-factor ANOVAs with temperature and pH as fixed factors. Prior to ANOVAs, homogeneity of variance was checked using Cochran's test. In case of significant effects of the investigated factors, Student–Newman–Keuls post hoc tests (SNK) were conducted (Quinn and Keough, 2002). Univariate analysis were carried out using GMAV (v.5) (University of Sydney). For the multivariate analysis of larval traits, morphological traits of all 75 larvae [POL, OL, BL; ASY and SHAPE (OL:BL)] were square

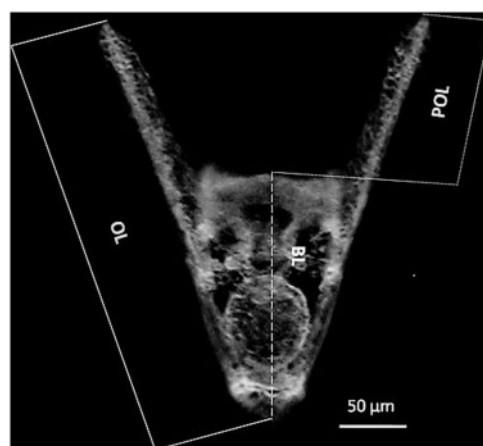


Figure 1. Morphological traits measured on *A. lixula* larvae: POL (post-oral length), OL (overall length) and BL (body length).

Table 1. Parameters for experiments with *A. lixula* to the 48 h echinopluteus in the six temperature-pH treatments.

Treatments		Measured		Calculated		
T (°C)	pH	T (°C)	pH _{NBS}	p_{CO_2} (μ atm)	Ω_{Ca}	Ω_{Ar}
20	8.2	20.32 \pm 0.06	8.17 \pm 0.02	417.56 \pm 3.35	5.12 \pm 0.20	3.34 \pm 0.16
20	7.9	20.21 \pm 0.06	7.88 \pm 0.02	920.79 \pm 5.26	2.91 \pm 0.23	1.90 \pm 0.19
24	8.2	24.34 \pm 0.05	8.17 \pm 0.01	427.48 \pm 1.03	5.60 \pm 0.07	3.58 \pm 0.06
24	7.9	24.52 \pm 0.08	7.86 \pm 0.06	1084.34 \pm 16.40	3.28 \pm 0.55	2.16 \pm 0.44
26	8.2	26.28 \pm 0.07	8.20 \pm 0.02	395.28 \pm 2.53	6.21 \pm 0.19	4.12 \pm 0.16
26	7.9	26.10 \pm 0.08	7.95 \pm 0.06	916.17 \pm 14.74	3.85 \pm 0.48	2.56 \pm 0.45

Experimental conditions [Temperature (°C) and pH (units)] and carbonate system speciation (p_{CO_2} , Ω_{Ca} , and Ω_{Ar}) calculated using pH_{NBS}, temperature, salinity (38), and total alkalinity (A_T 2500 μ mol/kg) for the six treatments (3 temperature \times 2 pH levels) (data are expressed in mean \pm standard error).

root transformed and displayed using an MDS (Multi-Dimensional Scaling) ordination on Euclidean distance matrix adding vectors of Pearson correlation. A PERMANOVA (multivariate analysis of variance based on permutation) was carried out on *A. lixula* larvae to examine significant effect of stressors (temperature and pH as fixed factors) and their interaction on the morphology pattern of larvae. Multivariate analysis was performed using PRIMER v6 software (Clarke and Warwick, 2001).

Results

Impacts of warming and acidification on larval growth

Temperature and pH had a significant effect on POL as single factors (Temp: $F_{2,149} = 95.73$, $P < 0.000$; pH: $F_{1,149} = 14.81$, $P < 0.001$) but there was no interaction between factors (Table 2). Larvae reared at 24°C and pH 7.9 had the longest POL (mean POL = 125.04 µm, SE = 2.92, $n = 25$) (SNK $20 < 26 < 24^\circ\text{C}$, Table 2; Figure 2).

For OL temperature and pH were both significant (Temp: $F_{2,149} = 21.64$, $P < 0.001$; pH $F_{2,149} = 4.47$, $P < 0.05$). There was also a significant effect “Temperature × pH” interaction ($P < 0.001$). The larvae reared at 24°C and 7.9 pH had the longest OL (226.37 µm, SE = 2.38, $n = 25$) (Table 2). Also, SNK a posteriori tests indicated significant differences in OL between temperatures, and among all pH treatments within temperatures. A positive effect of temperature in larvae reared at pH 8.2 was detected, indicating the presence of larger larvae in the higher temperature treatment compared with the control (SNK: $20^\circ\text{C}/8.2 < 24^\circ\text{C}/8.2 = 26^\circ\text{C}/8.2$; Table 2). Larvae reared at pH 7.9 had a shorter OL, but this effect was reduced at 26°C indicating that increased temperature provided a possible buffer effect (SNK: $26^\circ\text{C}/7.9 < 24^\circ\text{C}/7.9 = 20^\circ\text{C}/7.9$; Table 2).

For BL temperature ($F_{2,149} = 36.33$, $P < 0.001$ SNK: $20^\circ\text{C} < 24^\circ\text{C} < 26^\circ\text{C}$), but not pH had a significant effect (Table 2). There was however a significant “Temperature × pH” interaction on BL ($P < 0.01$). SNK tests indicated that larvae reared in pH 8.2 and 26°C had a significant longer BL (117.62 µm, SE = 1.17, $n = 25$) than those reared at 20 and 24°C (97.97 SE = 2.04, $n = 25$ and 111.96 µm, SE = 1.96, $n = 25$, respectively). Larvae reared at pH 7.9, had a shorter BL but increased temperature reduced this effect ($26^\circ\text{C}/7.9 < 24^\circ\text{C}/$

$7.9 < 20^\circ\text{C}/7.9$; Table 2). These data indicated the positive influence of temperature on larval growth at both pH levels used.

Temperature and pH had a significant effect on the percentage of larval ASY as single stressors (Temp: $F_{2,449} = 2.88$; $P < 0.01$; pH $F_{1,449} = 5.09$; $P < 0.02$). SNK tests indicated higher difference in the length of the two post-oral arms in larvae reared in the two cooler temperatures ($24^\circ\text{C} = 26^\circ\text{C} < 20^\circ\text{C}$) and at pH 8.2 than the other treatments (Table 3; Figure 3).

Temperature ($F_{2,449} = 74.52$; $P < 0.0001$) and to a lesser extent pH ($F_{1,449} = 4.46$; $P < 0.03$) had significant effect on SHAPE (OL:BL) with a significant “Temperature × pH” interaction. SNK tests showed that the OL:BL ratio at 24°C was the highest ($20^\circ\text{C} < 26^\circ\text{C} < 24^\circ\text{C}$) with larvae at 24°C showed a typical pluteus triangular shape (Table 3; Figures 3 and 4).

Multivariate analysis of larval traits

PERMANOVA analysis of the five morphological measurements (BL, OL, POL, ASY, and SHAPE) taken on each larva across six treatments, showed a significant effect of temperature and pH and a significant interaction between factors (Table 4).

The MDS ordination displays data with a partial vertical distribution by temperature from the bottom (20°C) to the upper (26°C) part of the graph. This distribution was most explained by the OL, POL, and SHAPE vectors (Pearson correlation > 0.75), in comparison with the BL and ASY traits (< 0.40). No pH clustering was evident within treatments (Figure 5).

Discussion

Increased temperature and reduced pH can have additive, both significant but no significant interaction, synergistic—increased stress greater than the sum of the effects, or antagonistic (decreased stress) effects on the developmental processes in marine invertebrates (Byrne and Przeslawski, 2013; Przeslawski, et al., 2015). Our study on the effects of simultaneous exposure to warming and lower pH on the development of *A. lixula* showed that larval growth was positively correlated with increased temperature and was negatively correlated with decreased pH. This was expected as in isolation, these stressors

Table 2. ANOVA results for the effects of factors Temperature and pH on POL, OL and BL data in the larvae of *A. lixula* in all treatments.

Factor	df	MS	F-value	P value	SNK
POL					
Temperature	2	162.55	95.73	0.00	20 < 26 < 24
pH	1	251.43	14.81	0.00	8.2 < 7.9
Temperature × pH	2	14.10	0.83	0.43	
Residual	144	1.69			
OL					
Temperature	2	20102.10	21.64	0.00	20 < 24 = 26
pH	1	4150.71	4.47	0.03	8.2 < 7.9
Temperature × pH	2	40028.39	43.08	0.00	20/8.2 < 24/8.2 = 26/8.2
Residual	144	929.07			26/7.9 < 24/7.9 < 20/7.9
BL					
Temperature	2	13438.48	36.3	0.00	20 < 24 < 26
pH	1	286.59	0.08	0.78	
Temperature × pH	2	2316.15	6.26	0.00	20/8.2 < 24/8.2 = 26/8.2
Residual	144	370.21			20/7.9 < 26/7.9 < 24/7.9

SNK post hoc test indicates significant difference whole treatments ($n = 25$).

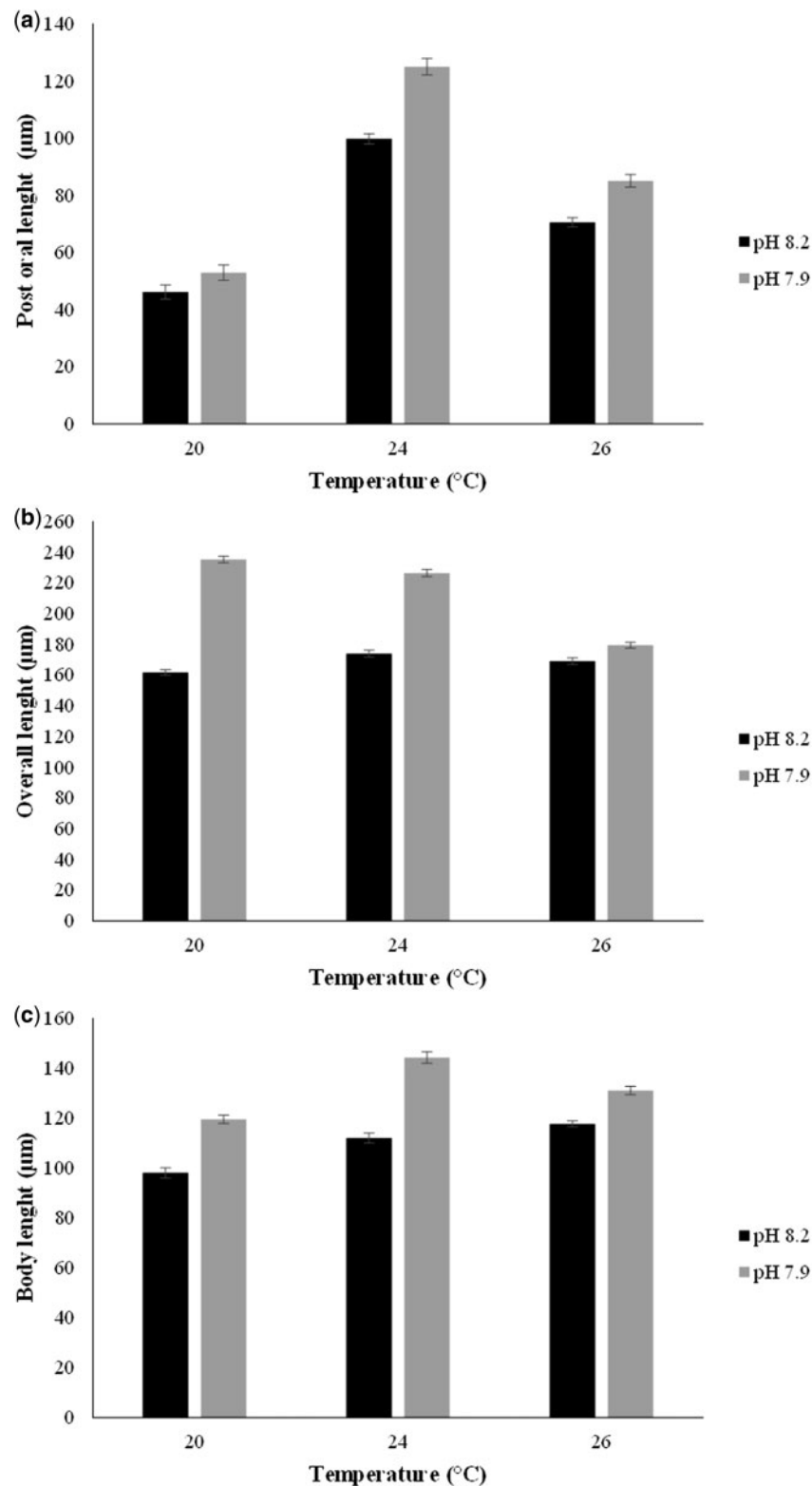


Figure 2. Mean (\pm SE) of POL (a), OL (b) and BL (c) in the *A. lixula* larvae in all treatments (3 Temperature \times 2 pH levels).

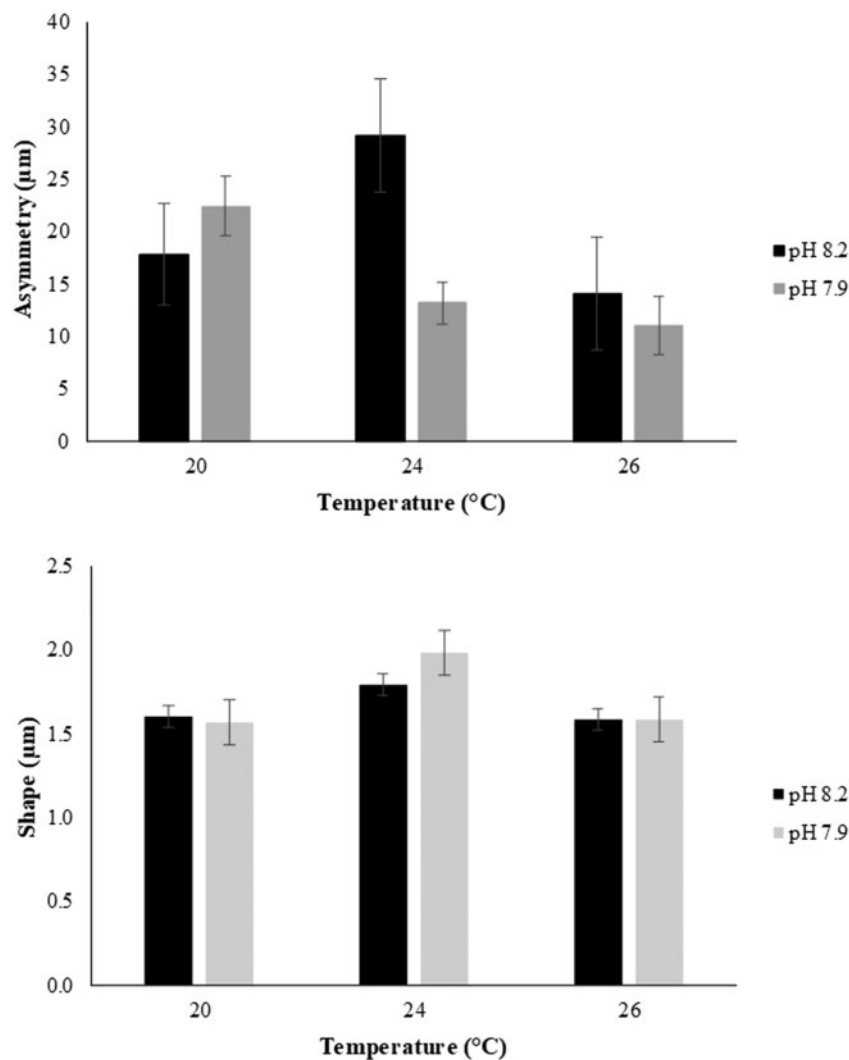
have stimulatory or stunting affects, respectively on the growth in sea urchin echinoplutei and other calcifying marine larvae (up to lethal limits) (McNeil *et al.*, 2004; Parker *et al.*, 2010; Arnberg *et al.*, 2013; Byrne *et al.*, 2013a, b). In the combined

treatments warming interacted antagonistically with acidification to reduce the negative effect of pH on decreased larval growth in *A. lixula*. This ameliorating effect of temperature was particularly evident in the high temperature (26°C) treatments.

Table 3. ANOVA results for the effects of factors Temperature and pH on ASY and Shape (OL:BL) data in the larvae of *A. lixula* in all treatments.

Factor	df	MS	F-value	P-value	SNK
ASY					
Temperature	2	13.58	2.88	0.06	24 = 26 < 20
pH	1	12.01	5.09	0.02	7.9 < 8.2
Temperature × pH	2	2.02	0.43	0.65	
Residual	444	1047.22			
Shape (OL:BL)					
Temperature	2	4561.00	74.52	0.00	20 < 26 < 24
pH	1	0.27	4.46	0.03	8.2 < 7.9
Temperature × pH	2	0.53	8.71	0.00	20/8.2 = 26/8.2 < 24/8.2
Residual	444	0.06			20/7.9 = 26/7.9 < 24/7.9

SNK post hoc test indicates significant difference whole treatments ($n = 75$).

**Figure 3.** Mean (\pm SE) of ASY (a) and Shape (b) data in the *A. lixula* larvae in all treatments (3 Temperature \times 2 pH levels).

Thus, our hypotheses that warming and acidification would alter larval body growth and that increased temperature would reduce the negative effects of acidification were supported. As expected, the larvae of *A. lixula* were resilient to warming and

developed over a relatively wide (6°C) temperature range. For the larvae of southern Mediterranean population investigated here, 24°C appears to approximate the optimal temperature for development.

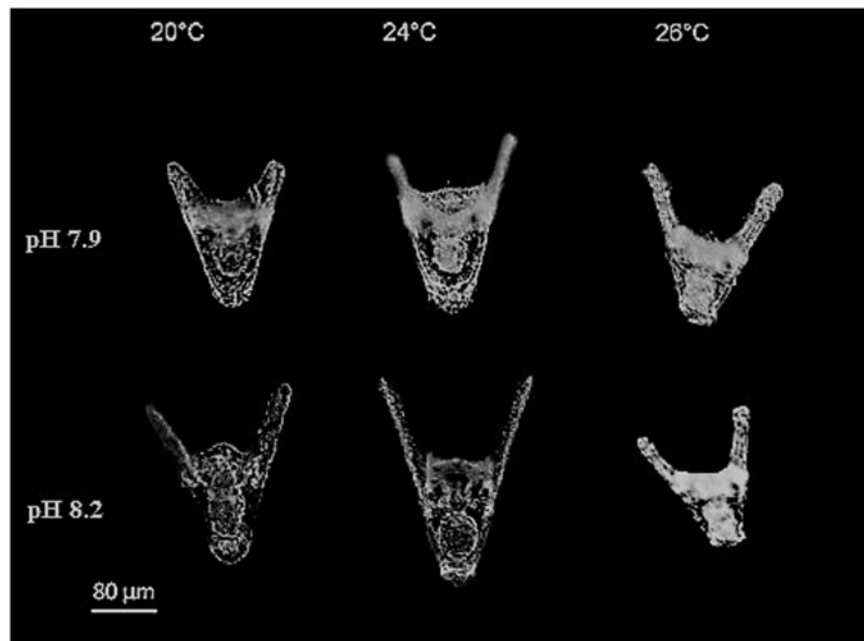


Figure 4. *Arbacia lixula* larvae reared for 48 h in three temperatures and two pH treatments.

Table 4. PERMANOVA results for the effects of Temperature and pH on larval size (BL, OL, POL) and body profile (asymmetry and shape ratio) of the *A. lixula* larvae for all treatments.

Source	df	MS	Pseudo-F-value	P-value (perm)	perms
Temperature	2	696.86	10 794	0.000	9999
pH	1	242.11	37 502	0.000	9999
Temperature × pH	2	106.31	16 467	0.000	9999
Residual	144	64559			

Analysis was based on Euclidian distances of normalized data, using 9999 random permutations (perms).

For sea urchin species with a broad latitudinal distribution, such as *A. lixula*, the concept of physiological differences among populations, with metabolic temperature compensation to different thermal regimes, suggests the potential for substantial plasticity to cope with global warming (Vernberg 1962; Palmer, 1994; Sokolova and Pörtner, 2001). Many studies showed that the optimal temperature for fertilization in sea urchins and other marine invertebrates is species and region specific based on the temperature at which the gametes develop (Olive, 1995; Sewell and Young, 1999; Byrne, 2011). The temperature at which the gametes are conditioned and the thermal window for fertilization success influences the optimal temperature for embryonic and larval development and so also exerts a major influence on the latitudinal distribution of species (Mita et al., 1984; Fujisawa, 1989; Hamdoun and Epel, 2007; Rahman et al., 2009; Byrne et al., 2013a, b; Pecorino et al., 2013). There is strong evidence that thermal acclimatization of the parents, particularly during oogenesis can dramatically shift the thermotolerance of the embryos of sea urchins and other invertebrates (O'Connor and Mulley, 1977; Fujisawa 1989, 1995; Johnson and Babcock 1994; Bingham et al., 1997; Rahman et al., 2009). Thus, the environmental history of the mother can influence the stress tolerance of progeny and this

is likely to be important in the difference in the optimal thermal regimes for *A. lixula* in the Northern (15–23°C) (Fenaux, 1968; Privitera et al., 2011; Wangenstein et al., 2013) and Southern (20–26°C, this study) Mediterranean Sea.

Fertilization and early development in a diverse suite of tropical and temperate sea urchins are robust to temperatures well above ambient and the increases expected from climate change (Byrne, 2011). This broad thermotolerance is likely to be conveyed by maternal factors loaded in the eggs during their development in response to environmental temperature and potentially include protective heat shock proteins that influence the upper thermal tolerance of progeny (Somero, 2002, 2010; Hammond and Hofmann, 2010). Our results show that *A. lixula* from the study population produces normal larvae across a 6°C temperature range (20–26°C) with an optimum at 24°C, similar to that found in a previous study of fertilization and early embryonic in this species (Gianguzza et al., 2014). The broad (6°C) thermal range for normal development of *A. lixula* at control pH is similar to that reported for other sea urchins that experience a broad thermal range across the spawning season (Delorme and Sewell 2013; Pecorino et al., 2013; Byrne, 2011).

Our data, together with the study of Wangenstein et al. (2013) in the NW Mediterranean and Privitera et al. (2011) from Ligurian population (North Tyrrhenian, near Catalan sub-basin) indicated that the thermal window for normal development in *A. lixula* could be as large $\approx 10^\circ\text{C}$ (from 16 to 26°C) with local differences influenced by the thermal history of the gametes prior to spawning. In NW Mediterranean *A. lixula* larvae develop at temperatures much lower than that used in this study (to 16°C) (Wangenstein et al., 2013). Conversely, Privitera et al. (2011) report that *A. lixula* larvae from Ligurian population (North Tyrrhenian, near Catalan sub-basin) did not develop below 18°C. The different thermal responses of development in these two neighbouring populations may be due to genetic differences (Wangenstein et al., 2012) that influence gamete performance. With respect to adult distribution

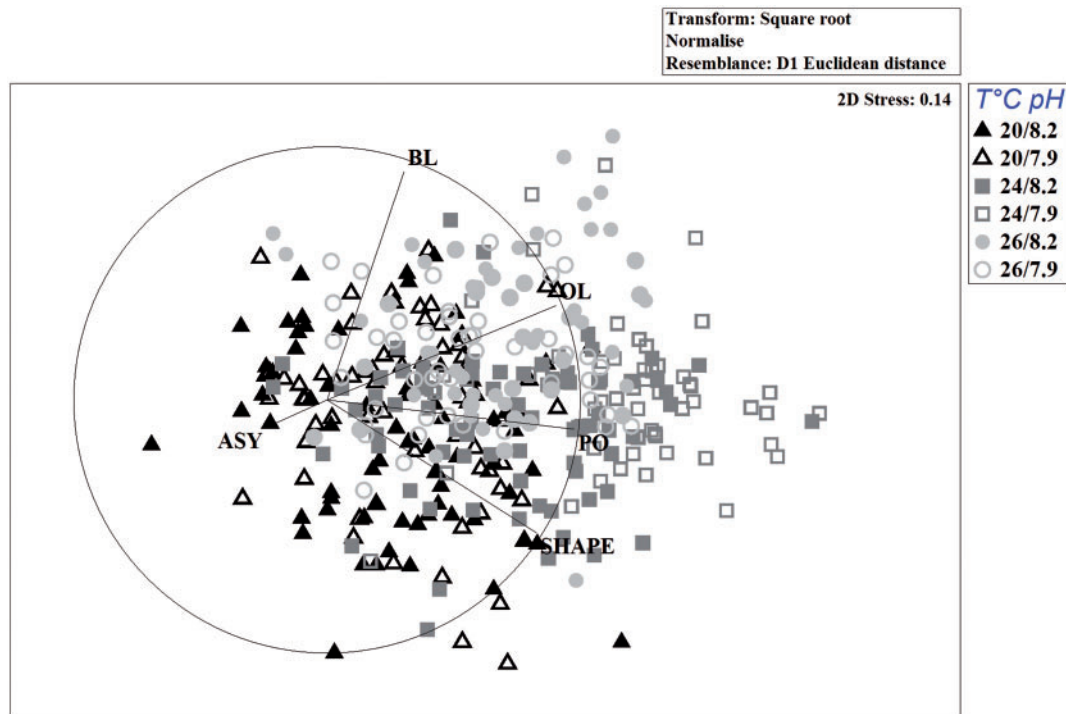


Figure 5. MDS ordination on morphological measures (POL, OL, and BL) and morphometric measures (ASY and SHAPE) in *A. lixula* larvae in the six treatments (3 Temperature \times 2 pH levels).

and larval development *A. lixula* appears to be both a cool and warm tolerant species with the thermal limits to successful development at any location are likely to be influenced by parental thermal experience, a phenotypic plastic response. In South Mediterranean region *A. lixula* may be living near its maximum thermal tolerance limit of larvae with sea temperatures up to 28°C in late summer. However, the larvae are not in the plankton at this time. The suggestion that different populations of *A. lixula* across Mediterranean regions have different developmental thermal tolerances needs to be addressed through determination of their respective thermal envelopes for development (e.g. Pecorino *et al.*, 2013; Hardy *et al.*, 2014; Byrne *et al.*, 2016).

Temperature is the major environmental factor that controls physiological and biological process in marine organisms (Hart and Scheibling, 1988; Hamdoun and Epel, 2007; Pörtner and Farrell, 2008; Byrne and Przeslawski, 2013). As characteristic of sea urchin development, larval size and the length of the skeletal elements increased with warming. There was also a clear response of body profile (SHAPE) to increased temperature indicating optimum at 24°C. These thermal effects on the shape of the echinoplutei have been found in other species and have important consequences for swimming and feeding of larvae (Soars *et al.*, 2009; Byrne *et al.*, 2013a, b; Hardy *et al.*, 2014). The interaction between temperature and pH is evident in OL and BL measures. In the high-temperature treatment (26°C) there was a buffering effect of warming—reducing the deleterious effects of pH on growth of BL than OL. Thus, the larvae in 26°C/low pH treatments and the control larvae reared at 24°C were similar in size. The POL data also indicated that 24°C was optimum, but this trait did not exhibit a significant interaction between stressors. The buffering effect of warming reducing the deleterious effects of pH on OL is also reported for the echinoplutei of the tropical species

Tripneustes gratilla where +3°C, ameliorated the negative effects of acidification (Sheppard Brennan *et al.*, 2010). Larval length size of *A. lixula* as indicated by the OL trait shows a strong correlation with temperature despite decreased pH. Our results indicated that the influence of increased temperature at near-future levels reduced the negative effect of acidification on the calcification response of *A. lixula*, as also seen in other invertebrate larvae and coral calcification (Byrne *et al.*, 2013a, b), although this likely to incur an energetic cost. As suggest by Chan *et al.* (2011) the morphological changes in larvae were coordinated to maintain swimming performance, an ecologically important function.

Species that have a broad distribution like *A. lixula* (~30° of latitude) (Lessios *et al.*, 2012; Wangenstein *et al.*, 2012) appear best able to avail of the new opportunities provided by ocean warming than sympatric species with a similar reproductive mode (e.g. Hardy *et al.*, 2014). However, the co-occurring sea urchin *Paracentrotus lividus* also has a broad distribution (~30° of latitude) (Boudouresque and Verlaque, 2001), but has narrow developmental thermal tolerance range in Mediterranean populations (18–22°C) and this species has not expanded its range (Pedrotti and Fenaux, 1992; Spirlet *et al.*, 1998; Shpigel *et al.*, 2004; Moulin *et al.*, 2011; Privitera *et al.*, 2011; Garcia *et al.*, 2015b). Spawning of *P. lividus* in the Mediterranean coincides with SST between 16 and 20°C, temperatures considered to be optimal for fertilization and development of this species, with impaired gametogenesis at 24°C (Spirlet *et al.*, 2000). It seems likely that *P. lividus* will have a lower reproductive as the climate warms. In addition, commercial harvest has resulted in a large-scale decline in the abundance of *P. lividus* in the Mediterranean (Guidetti, 2004; Gianguzza *et al.*, 2006; Pais *et al.*, 2012). This together with the greater sensitivity of *P. lividus* to warming may release *A. lixula* from competition, thus facilitating the success of this species.

In parallel with having a broad thermotolerance, *A. lixula* is also more robust to pH variation than *P. lividus* as shown in the Mediterranean CO₂ vent systems at Vulcano, where *A. lixula* was more prevalent at higher pCO_{2sw} sites compared with *P. lividus* (Calosi et al., 2013; Bray et al., 2014). For this site it is suggested that *A. lixula* is better able to adjust its acid–base balance, ionic regulation in naturally fluctuating pCO_{2sw} conditions than the co-occurring *P. lividus* (Calosi et al., 2013). This capacity of *A. lixula* to maintain acid–base homeostasis appears to be due to the naturally higher levels of protein in the coelomic fluid of *A. lixula* (Small et al., 2015). However at the Ischia vent system, Kroeker et al. (2013) noted a decline in both species along a pH gradient. This difference in the response of these species at the two vent systems in the Mediterranean may be due to location specific effects and potentially trophic considerations as *A. lixula* and *P. lividus* differ greatly in their diet. *A. lixula* is omnivorous with a high protein (carnivory) diet while *P. lividus* is herbivorous (Wangensteen et al., 2011; Agnetta et al., 2013).

Climate warming acting synergistically with overfishing may allow a shift from a ubiquitous presence in Mediterranean rocky reefs of *P. lividus* and *A. lixula* to a dominant presence of *A. lixula*. If *A. lixula* increases its densities in the foreseeable future, this will have serious consequences for the Mediterranean ecosystem diversity and function. As *A. lixula* is less prone to predation than *P. lividus*, and is not fished, the thermal and pH tolerance of this species may establish a positive feedback to stabilize and maintain the barren grounds in rocky littoral ecosystems (Bonaviri et al., 2011).

It is clear that the warming of the Western Mediterranean is creating an environment increasingly favourable for successful reproduction and development of *A. lixula* (Privitera et al., 2011; Wangenstein et al., 2012; Gianguzza et al., 2014). Across the Mediterranean SST ranges from 9.7 to 17.7°C in winter, 15.8 to 22.1°C in spring, 20.8 to 28.3°C in summer and 15.1 to 23.4°C in autumn (Shaltout and Omstedt, 2014), with contrasting temperatures in the northern and southern Tyrrhenian sub-basin.

Despite the complex circulation and contrasting temperatures across the Mediterranean Sea, *A. lixula* is widely distributed reflecting its broad developmental thermotolerance, and as shown here, at levels overlapping with the warming conditions projected for 2100. It is likely that *A. lixula* adjusts its reproductive biology (from fertilization to larvae development) to local environmental conditions across Mediterranean Sea and its planktonic phase assures population connectivity between sub-basin populations.

The resilience of *A. lixula* in a warming environment is likely to be a key driver of ecological structure of Mediterranean rocky subtidal habitat. A pressing challenge in ecology is to understand and predict the effects of global warming on marine realm. The global increase in mean temperature is expected to profoundly alter temperate communities, with cascading effects for the biodiversity and function of coastal ecosystems (Burrows et al., 2011; Vergés et al., 2014; Garcia Molinos et al., 2016). Future research should focus on the effects of multiple stressors on the recruitment and adult survival of *A. lixula* to have a more complete understanding of potential changes in the population dynamics of this species and potential consequences for regional benthic community structure, in relation with global changes.

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