



Temperature modulates the response of the thermophilous sea urchin *Arbacia lixula* early life stages to CO₂-driven acidification



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ABSTRACT

The increasing abundances of the thermophilous black sea urchin *Arbacia lixula* in the Mediterranean Sea are attributed to the Western Mediterranean warming. However, few data are available on the potential impact of this warming on *A. lixula* in combination with other global stressors such as ocean acidification. The aim of this study is to investigate the interactive effects of increased temperature and of decreased pH on fertilization and early development of *A. lixula*. This was tested using a fully crossed design with four temperatures (20, 24, 26 and 27 °C) and two pH levels (pH_{NBS} 8.2 and 7.9). Temperature and pH had no significant effect on fertilization and larval survival (2d) for temperature <27 °C. At 27 °C, the fertilization success was very low (<1%) and all larvae died within 2d. Both temperature and pH had effects on the developmental dynamics. Temperature appeared to modulate the impact of decreasing pH on the % of larvae reaching the pluteus stage leading to a positive effect (faster growth compared to pH 8.2) of low pH at 20 °C, a neutral effect at 24 °C and a negative effect (slower growth) at 26 °C. These results highlight the importance of considering a range of temperatures covering today and the future environmental variability in any experiment aiming at studying the impact of ocean acidification.

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1. Introduction

Since the beginning of the industrial revolution and the extensive use of fossil fuels, global atmospheric CO₂ concentration has increased from 280 to 380 ppm and is expected to double by 2100 with well described consequences for climate (global warming, increase in extreme events frequency, etc.). The ocean represents a major sink and absorbs half of the excess of CO₂. This continued uptake of CO₂ alters the carbonate chemistry of the ocean and increases the concentration of hydrogen ions, thereby reducing pH, a phenomenon called ocean acidification (Caldeira and Wickett, 2003). The mean global surface temperature has increased 0.76 °C in the past 150 years and is predicted to rise an additional 1.1°C–6.4 °C by the end of the twenty-first century (IPCC, 2007;

Fabry et al., 2008). Ocean acidification scenarios project a ΔpH = –0.3 to –0.5 units by the end of the century.

The influence of temperature and pH on echinoderms is well documented as single stressors (e.g. Dupont et al., 2010a; Byrne, 2011; Dupont and Thorndyke, 2013). Temperature impacts metabolisms and modulates performance (Byrne, 2011). Direct impacts of ocean acidification on sea urchins are mostly negative but sub-lethal. These include slower somatic and gonadal growth and reflect a shift in energy budgets linked to additional costs for extracellular pH (pHe) and intracellular pH (pHi) regulations rather than direct impact on calcification (Dupont and Thorndyke, 2013). However, interaction between temperature and ocean acidification was only considered in a limited number of studies (Sheppard Brennard et al., 2010; Byrne et al., 2009, 2010a,b; 2011; Caldwell et al., 2011; Catarino et al., 2012a; Ericson et al., 2012; Foo et al., 2012). From this limited dataset, it appears that interaction between temperature and ocean acidification is complex from temperature being the main driver of change to temperature amplifying or diminishing the negative effects of ocean acidification. For example, in adult *Paracentrotus lividus* oxygen uptake was increased under ocean acidification at 10 °C but not at 16 °C (Catarino et al., 2012a). Warming seemed to diminish the negative

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effect of acidification on *Tripneustes gratilla* larval growth (Sheppard Brennard et al., 2010). Pörtner and Farrell (2008) developed a theoretical framework to predict the combined impact of temperature and ocean acidification. All organisms live within a limited range of body temperatures, due to optimized structural and kinetic coordination of molecular, cellular, and systemic processes, and functional constraints result at temperature extremes. It is hypothesized that synergistic stressors like ocean acidification have the potential to narrow these thermal windows. This theoretical framework highlights the fact that response to ocean acidification can be highly dependent upon thermal conditions. This can explain apparently conflicting results. For example, warming may lead to increased resilience to ocean acidification in experiments done at the lower end of the optimal temperature range of the species, but can enhance sensitivity when an experiment is done close to the upper limit of the thermal tolerance. It is then critical, in any experimental design, to cover the natural range of present and future thermal ranges naturally experienced by any given species to fully understand the potential impact of ocean acidification.

Research on global changes needs to focus on species which play dominant structuring roles in ecosystems (e.g. herbivores; habitat-forming species; Russell et al., 2012). Sea urchins are excellent candidates. They are important grazers that structure habitats and affect temporal dynamics and ecosystem functions (e.g. through trophic cascades; Sala et al., 1998; Bonaviri et al., 2012; Hereu et al., 2012).

The black *Arbacia lixula* is a common inhabitant of shallow-water hard grounds throughout the Mediterranean Sea. It is also found on the other side of the Atlantic, but only in the Southern Hemisphere, off the coast of Brazil (Gianguzza and Bonaviri, 2013). It is currently one of the most abundant echinoids in shallow rocky habitats of the southern Mediterranean (Guidetti and Dulčić, 2007). This species has a considerable trophic plasticity, ranging from omnivory to strict carnivory (Wangensteen et al., 2011; Agnetta et al., 2013) and its scraping predatory behaviour can play a dominant role in driving switches between one complex state, dominated by a stratified assemblage of several erect macroalgae, to a simpler one dominated by few encrusting algae: the so-called 'barren ground' (Bonaviri et al., 2011; Wangensteen et al., 2012; Agnetta et al., 2013). New and increasing evidence suggests that on-going warming of the Western Mediterranean results in an environment increasingly favourable for the reproduction and development of *A. lixula* (Francour et al., 1994; Guidetti and Dulčić, 2007; Gianguzza et al., 2011; Privitera et al., 2011; Lessios et al., 2012; Wangensteen et al., 2012).

Considering the great colonizing potential shown by this species, including the ability to cross trans-oceanic barriers to gene flow (Wangensteen et al., 2012), and the massive potential impact of its grazing on coastal ecosystems, it is critical to evaluate how this potential will be modulated by near-future changes such as ocean warming and acidification. In the Western Mediterranean, the planktotrophic *A. lixula* larvae occur in the water column between Spring and Autumn and can be exposed to the full range of the temperature natural variation (15–24 °C; Fenaux, 1968; Pedrotti, 1993).

The present study aims to investigate the combined impacts of ocean warming and acidification on the early life-history stages of the sea urchin *A. lixula*. The impact of ocean acidification and warming was tested using a fully crossed design with four temperatures (20, 24, 26 and 27 °C) and two pH levels (pH_{NBS} 8.2, present average pH and pH_{NBS} 7.9, pH projected by 2100). The selected temperatures cover the upper part of today and near-future environmental variability. Based on Pörtner and Farrell (2008), we predicted an overall negative impact of pH on the tested parameters (fertilization, larval survival and developmental rate).

2. Materials and methods

2.1. Animal collection and maintenance

Adult *A. lixula* (test diameter of 35–45 mm) were collected in a shallow rocky shore (3–5 m) along the coast of Palermo (38°11'45"N-013°14'58"E) during the peak reproductive season from May to September 2010 (G. Visconti, unpublished data). Collected animals were brought to the laboratory and were kept prior to the experiment for less than 24 h in recirculating tanks at constant temperature (20 °C) and pH_{NBS} (8.2).

2.2. Fertilization and larval culture

To avoid male-female incompatibility and mimic field fertilization success, fertilization was conducted with gametes pooled from multiple males and females. Six females and six males were used. Gonads were dissected and rinsed few times in filtered seawater (FSW; Millipore filter 0.45 µm). Eggs were collected and mixed. Sperm was collected, mixed and kept dry before use. Replicates of 900 ml of mixed eggs dilution (1500 eggs/L) were pre-incubated in experimental FSW for 15 min and then mixed 200 µl of dry sperm (final concentration of 3000 sperm/mL) in each replicate for fertilization. During larval culture, there was no food supply and the larvae subsisted on their own reserves.

2.3. Experimental treatments

Eight different treatments were tested: 4 temperatures (20°, 24°, 26°, 27 °C) × 2 pH (8.2 and 7.9). Each treatment was replicated 6 times. The seawater pH was adjusted by bubbling CO₂ until the target pH was reached. Cultures were then kept in closed bottles for 2d and gently mixed using motor driver paddles. Temperature was maintained using an immersion heater. Temperature and pH was measured 5 times over the course of the experiment. pH was measured using a pH meter (Crison GLP21) calibrated with NBS buffers. The carbonate system speciation (pCO₂, Ω_{ca} and Ω_{ar}) was calculated from pH_{nbs}, temperature, salinity (38) and alkalinity (A_T = 2.5 mM; Rivaro et al., 2010) using CO₂SYN (Lewis and Wallace, 1998) with dissociation constants from the study by Mehrbach et al. (1973) refitted by Dickson and Millero (1987).

2.4. Measured parameters

Three 1 ml samples were collected in each replicate (48 cultures) at 4 h, 12 h, 24 h, 36 h and 48 h and fixed with few drops of formaldehyde (10% in FSW). Eggs, embryos and pluteus larvae were counted in each sample and fertilization success (%) after 4 h was calculated for each replicate as the ratio between successful fertilization (embryos with 2–8 cells) and unfertilized eggs. Mortality after 48 h was calculated for each replicate as:

$$\text{Mortality}(\%) = 100 \times [1 - (\text{larval density at 48h} / \text{initial density})]$$

For each replicate, the % of pluteus larvae ($100 \times [\text{number of pluteus larvae} / \text{number of embryos and larvae}]$) was calculated at 12 h, 24 h, 36 h and 48 h. A limited number of larvae were scored for each replicate and time point (<50) and to improve the power of this parameter, the replicates were merged for each treatment. The dynamic of development was characterized by the inflection point of a Gompertz equation of an asymmetrical growth curve estimated by least-square method from % of pluteus larvae and time (Krönström et al., 2007).

2.5. Statistics

Each mean value is expressed with its standard error of mean (mean \pm SEM). Two factor model ANOVA was used to test the impact of treatment (fixed, pH and temperature) on fertilization success and mortality. When relevant, Scheffe's post-hoc test was used to test difference between treatments. The Shapiro–Wilk test (1965) was used to confirm that the data were normally distributed and the Levene test was used to confirm that variances were homogenous. All data were analysed using SAS/STAT software.

3. Results

3.1. Seawater chemistry

Temperature and pH_{nbs} were maintained at the target level over the course of the experiment (Table 1). Significant difference in temperature were maintained between the temperature treatments (ANOVA 3, $F = 236.27$, $p < 0.0001$; temperature, $F = 3306.79$, $p < 0.0001$) with no significant differences between nominal pH ($F = 0.02$, $p = 0.88$) or replicates ($F = 0.38$, $p = 0.93$) within a same nominal temperature. For pH, our treatments were control seawater (nominal pH = 8.2, pH_{nbs} = 8.16 ± 0.01 ; pCO₂ = 511.00 ± 8.85 μ atm, $\Omega_{ca} = 4.77 \pm 0.07$, $\Omega_{ar} = 3.08 \pm 0.04$) and elevated pCO₂ (nominal pH = 7.9, pH_{nbs} = 7.90 ± 0.01 ; pCO₂ = 1053.41 ± 34.32 μ atm, $\Omega_{ca} = 2.91 \pm 0.07$, $\Omega_{ar} = 1.88 \pm 0.05$). Significant difference in pH_{nbs} were maintained between the pH treatments (ANOVA 3, $F = 12.22$, $p < 0.0001$; pH, $F = 255.86$, $p < 0.0001$) with no significant differences between nominal temperature ($F = 0.98$, $p = 0.46$) or replicates ($F = 2.51$, $p = 0.06$) within a same nominal pH.

3.2. Impact of pH and temperature on fertilization success

Fertilization success of *A. lixula* at 4 h was high (>85%) at 20 °C and decreased with increasing temperature (68% at 24 °C, 55% at 26 °C and <1% at 27 °C; Fig. 1). pH had no significant effect on fertilization success (ANOVA 2, model: $F = 14.01$, $p < 0.001$; pH: $F = 0.07$, $p = 0.79$) with no interaction with temperature (pH \times temp: $F = 0.05$, $p = 0.98$). Temperature had a significant effect on fertilization success (temp: $F = 32.61$, $p < 0.001$), 27 °C (<1% fertilization) being significantly different than all other temperatures (Scheffe's post-hoc test, $p < 0.05$; >50% fertilization >50% fertilization).

3.3. Impact of pH and temperature on mortality

Mortality at 48 h was highly variable among treatments (between 11 and 100%; Fig. 2). However, only temperature has a significant effect on mortality (model: $F = 5.95$, $p < 0.001$; temp:

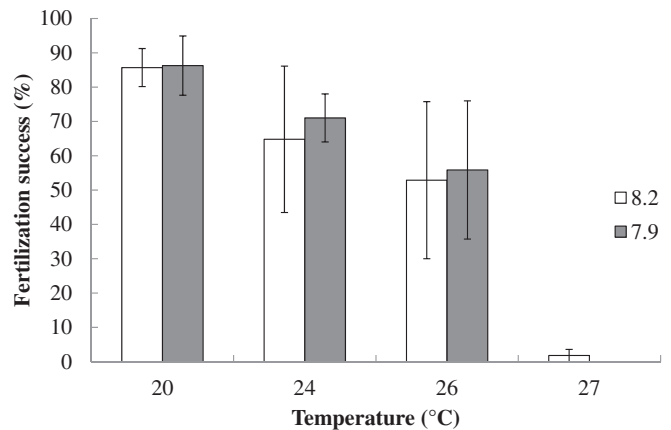


Fig. 1. Impact of pH and temperature on *A. lixula* fertilization success at 4 h.

$F = 11.18$, $p < 0.001$), 27 °C with 100% mortality was significantly different from all other temperatures (Scheffe's post-hoc test, $p < 0.05$). No significant effect of pH was observed (pH: $F = 3.72$, $p = 0.06$) with no interaction with temperature (pH \times temp: $F = 1.33$, $p = 0.28$).

3.4. Impact of pH and temperature on developmental rate

The inflexion point (Ip in h) from a Gompertz growth model between time (h) and % of pluteus larvae was calculated as a proxy for developmental rate. At pH 8.2, development to the pluteus stage was faster in warmer temperature (Ip increasing from 19.62 h at 20 °C to 11.20 h at 26 °C; Fig. 3). The pH effect of developmental rate was dependent of the temperature. At 20 °C, the development was faster at pH 7.9 (Ip = 9.57 h) compared to pH 8.2 (Ip = 19.62 h; Fig. 3A). At 24 °C, the development was similar at both pH (Ip = 14 h at pH 8.2 and Ip = 12.33 at pH 7.9; Fig. 3B). At 26 °C, lower pH had a negative effect on developmental rate leading to a slower development (Ip = 25.45) at pH 7.9 compared to pH 8.2 (Ip = 11.20; Fig. 3C). The mortality rate at 27 °C was 100% and it was therefore not possible to calculate a developmental rate.

4. Discussion

4.1. Physiological consequences

4.1.1. Impact of ocean acidification and warming on fertilization

Our results showed that fertilization success in *A. lixula* – measured as the number of cleaving embryos after 4 h – was robust to both ocean acidification (Δ pH = -0.3) and warming for temperature <27 °C. At higher temperature (27 °C), fertilization success

Table 1
Seawater carbonate chemistry during the experiment. The carbonate system speciation (pCO₂, Ω_{ca} and Ω_{ar}) was calculated from pH_{nbs}, temperature, salinity (38) and alkalinity ($A_T = 2.5$ mM; Rivaro et al., 2010) using CO2SYS (Lewis and Wallace, 1998).

Nominal		Measured		Calculated		
Temp [°C]	pH	Temp [°C]	pH _{nbs}	pCO ₂ (μ atm)	Ω_{ca}	Ω_{ar}
20	8.2	20.35 \pm 0.05	8.16 \pm 0.01	497.96 \pm 16.52	4.34 \pm 0.10	2.77 \pm 0.06
20	7.9	20.25 \pm 0.04	7.89 \pm 0.01	1000.54 \pm 27.33	2.51 \pm 0.06	1.60 \pm 0.04
24	8.2	24.39 \pm 0.04	8.15 \pm 0.01	518.63 \pm 15.11	4.79 \pm 0.10	3.09 \pm 0.06
24	7.9	24.48 \pm 0.07	7.86 \pm 0.02	1166.19 \pm 76.11	2.73 \pm 0.12	1.76 \pm 0.08
26	8.2	26.10 \pm 0.08	8.16 \pm 0.01	509.66 \pm 17.22	5.14 \pm 0.12	3.23 \pm 0.08
26	7.9	26.04 \pm 7.97	7.97 \pm 0.02	895.04 \pm 0.02	3.55 \pm 0.13	2.30 \pm 0.13
27	8.2	26.90 \pm 0.07	8.14 \pm 0.01	544.74 \pm 14.99	4.97 \pm 0.08	3.23 \pm 0.05
27	7.9	27.21 \pm 0.11	7.83 \pm 0.03	1264.36 \pm 87.81	2.77 \pm 0.18	1.80 \pm 0.12

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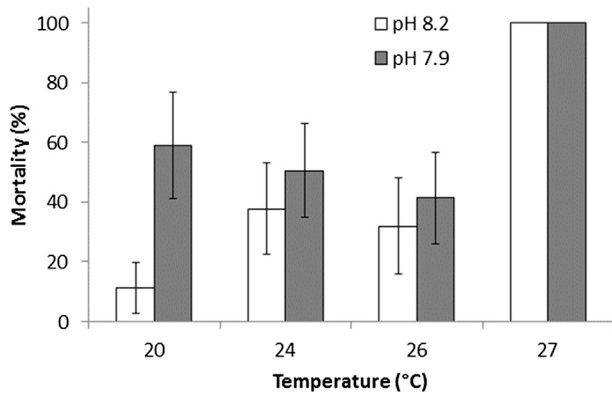


Fig. 2. Impact of pH and temperature on *A. lixula* mortality 48 h post-fertilization.

was dramatically reduced at less than 1%. The impact of ocean acidification ($\Delta\text{pH} < -0.7$), alone (11 articles) or in combination with increased temperature (6 articles), on gametes and fertilization was tested on 15 other echinoderm species. In 7 species, ocean

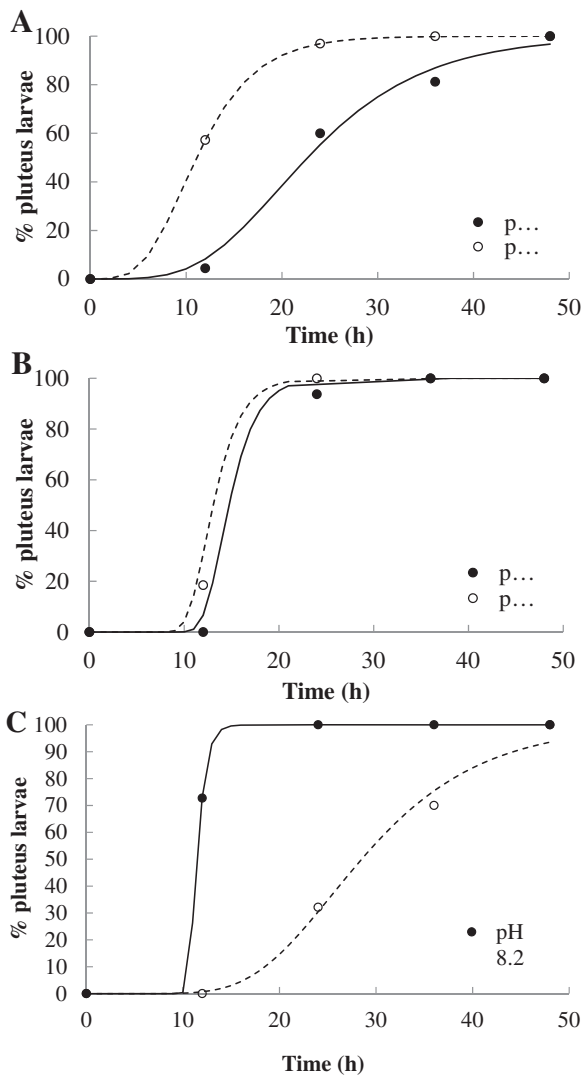


Fig. 3. Impact of pH on the % of pluteus larvae through time at 3 different temperature (A, 20 °C; B, 24 °C and C, 26 °C). A Gompertz growth model was used to model the relationship between time (d) and % of pluteus larvae.

warming and acidification had no effect of fertilization success (*Centrostephanus rogersii*, Byrne et al., 2010b; *Echinometra mathaei*, Kurihara and Shirayama, 2004; Kurihara et al., 2004; *Heliocidaris tuberculata*, Byrne et al., 2010b; *Hemicentrotus pulcherrimus*, Kurihara and Shirayama, 2004; Kurihara et al., 2004; *Meridiastra calcar*, Nguyen et al., 2012; *Patiriella regularis*, Byrne et al., 2010b; *T. gratilla*, Byrne et al., 2010b). Ocean acidification reduced fertilization success in *Arachnoides placenta* (Gonzalez-Bernat et al., 2012) and *Strongylocentrotus franciscanus* (Reuter et al., 2011) and in two other species, ocean acidification had no negative effect on fertilization except at low sperm concentration (*Odontaster validus*, Gonzalez-Bernat et al., 2013) or when combined with increased temperature (*Sterechinus neumayeri*, Ericson et al., 2010, 2012). Mixed responses from negative to positive were observed in *P. lividus* (Moulin et al., 2011; Martin et al., 2011) and *Heliocidaris erythrogramma*. Using similar pH changes and working on the same species (*H. erythrogramma*), Byrne et al. (2009; 2010a,b) showed no effect on fertilization while Havenhand et al. (2008) showed a negative effect. This may partly reflect difference in experimental design (polyandry vs single male-female crosses, sperm concentration, stability of pH, use of different sperm:egg ratio, sperm-egg contact time etc.; e.g. Reuter et al., 2011) but also individual variability in reproductive success (Schlegel et al., 2012). Our results which showed no effect of ocean acidification on fertilization success were then consistent with most of the published data. However, we only considered a high sperm-egg ratio (2000:1) and long sperm-egg contact time and our results may underestimate this impact. Extreme temperature (27 °C) was inhibiting fertilization (<1%). A similar impact was observed in *H. erythrogramma* (Byrne et al., 2009). As a consequence, *A. lixula* fertilization might be sufficiently resilient to near-future ocean warming and acidification although it should be impaired during extreme warming events. Such results emphasise the pressing need to identify approaches that can rapidly assess the degree of stress experienced by populations, integrate the effects of multiple stressors and predict the likely outcome for population persistence (Sokolova et al., 2012; Sarà et al., 2012).

4.1.2. Impact of ocean acidification and warming on larval survival

In the majority of tested species (9 out of 13), ocean acidification had no effect on larval survival (see Dupont and Thorndyke, 2013 for review). In the other 4 species, the impact was an increased mortality (*Arachnoides placenta*, Gonzalez-Bernat et al., 2012; *Odontaster validus*, Gonzalez-Bernat et al., 2013; *Patiriella regularis*, Byrne et al., 2013) with a 100% mortality within 7 days observed in the brittlestar *Ophiothrix fragilis* (Dupont et al., 2008). Our results showed that ocean acidification, alone or in combination with elevated temperature (<27 °C) do not increase the larval mortality. At the highest temperature (27 °C), all larvae experienced increased mortality, dying in only 3d. Present result is consistent with that observed in the larval sea star *Patiriella regularis* (Byrne et al., 2013) and it represents a further support on the fact that the temperature is the main factor limiting fertilization and larvae survival as recently showed by many studies on thermal tolerance limits of marine ectotherms (Kooijman, 2010 for review). In our experimental species, larvae were subjected to an evident physiological tipping point around 27 °C. Such a result mirrored the physiological tolerance limits of *A. lixula* larvae resembling the western Mediterranean pelagic thermal habitat where it lives. Temperature of water masses of the first infralittoral zone (from subsurface to the first thermocline at about 10–13 m) is on average indeed around 24–26.5 °C in Southern MED, rarely exceeding the 27 °C unless for a few hours during warmer days. *A. lixula* larvae could moreover live very close to the edge of their metabolic machinery functioning (Sarà et al., 2011). As a consequence, warming in a context of

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climate change or heating waves (e.g. Garrabou et al., 2009) may lead to mass mortalities of sea-urchin's larval reservoir as shown for other benthic organisms (Cerrano et al., 2000).

4.1.3. Impact of ocean acidification and warming on larval growth

Growth rate is a more sensitive endpoint to environmental changes and one of the most documented effects of ocean acidification on larval stages is a delay in development. For example 16 out of the 19 tested species of echinoderm's larvae showed a delay in development when raised in ocean acidification conditions (see Dupont and Thorndyke, 2013 for review). Temperature has a very well described positive effect on growth rate up to the optimum growth rate while extreme increasing temperatures induce delay in development (Duarte, 2007). Only 3 studies studied the combination between warming and acidification on echinoderm larvae. In seastar *Meridiastra calcar* larval growth rate decreased while temperature increased although there was not any effect from acidification treatment (Nguyen et al., 2012). Based on the theoretical framework developed by Pörtner and Farrell (2008) it was predicted that (i) response to ocean acidification is modulated by temperature and, (ii) ocean acidification can only drive negative response in animals. For example, it is predicted that warming can increase resilience to ocean acidification on the cold side of a species optimal temperature, but enhances sensitivity when a species

experiences both drivers close to its upper limits of thermal tolerance. This is supported by most of the work published on the impact of ocean acidification on echinoderm larvae with two noticeable exceptions as follows: (i) a +3 °C warming minimized the negative effect of acidification on larval growth in the larvae of the sea urchin *T. gratilla* (Sheppard Brennan et al., 2010) and (ii) larval stages of the sea star *Crossaster papposus* increased their growth when exposed to ocean acidification (Dupont et al., 2010b). Our results challenged the hypothesis that ocean acidification can only have a negative impact on larval development. Indeed, temperature modulates the impact of decreasing pH on the developmental rate with a positive effect (faster growth) under ocean acidification conditions at 20 °C, a neutral effect at 24 °C and a negative effect (slower growth) at 26 °C. This apparent paradox can be solved thanks to a good mechanistic understanding of the impact of both temperature and ocean acidification on larval physiology and energy budget (Fig. 4). Both increased temperature (e.g. Peck and Prothero-Thomas, 2002) and pH (Stumpp et al., 2011, 2012) induce an up-regulation of larval metabolism. An increased metabolism under non-limiting energy conditions can translate into an increased scope for growth. This explains the well-documented positive effect of temperature on growth rates since temperature often leads to an increased metabolism and an increase in food uptake (e.g. Podolsky and Emler, 1993). This can also

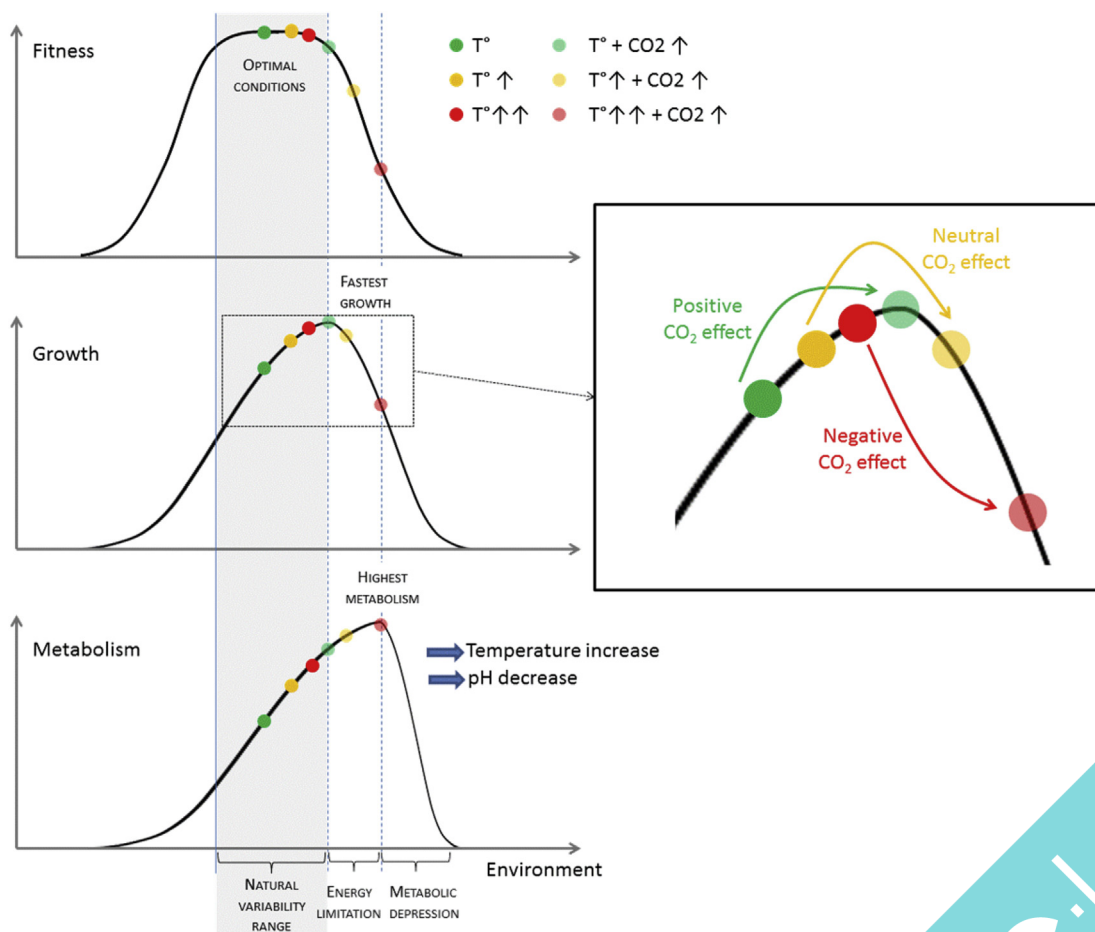


Fig. 4. Theoretical predictive model showing the relationship between echinoderm larval fitness, growth and metabolism under different environmental challenges. Optimal conditions are defined by the range of metabolism and growth without negative consequence for fitness (survival). An increase in metabolism is first associated with an increased growth till the point of energy limitation. Then, any increase in metabolism lead to a reduced scope for growth and a decreased growth. Both ocean warming and acidification are inducing an increase in metabolism (shift to the right on the metabolic curve) till the point of metabolic depression and lethality. Ocean acidification can then lead to a positive effect (not energy limiting conditions, green dots) or a negative effect (energy limiting conditions, red dots) on growth rates depending on the baseline metabolism. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

explain the positive effect of ocean acidification on the larvae of the sea star *Crossaster papposus* (Dupont et al., 2010a,b,c). It was hypothesized that the lecithotrophic life-history strategy (production of few energy rich eggs) allowed an increased growth following an increase in metabolism. On the other hand, under limiting energy conditions, an increased metabolism is associated with a reduced scope for growth as demonstrated in sea urchin larvae exposed to ocean acidification conditions (Stumpp et al., 2011). We can also hypothesize that under extreme stress, larvae are unable to compensate for the environmental challenge through increased metabolism and experience metabolic depression (Pörtner and Farrell, 2008). In theory, different combinations of increased temperature and pH can then lead to different sublethal biological responses. (i) If the baseline metabolism is far from its optimal (e.g. pH 8.2/20 °C in our experiment), the larvae is not energy limited and an increase in metabolism (e.g. increased temperature or decreased pH) can lead to an increased growth rate; (ii) If the baseline metabolism is closer to its optimal (e.g. pH 8.2/26 °C), any increase in metabolism will lead to a reduced scope for growth and lead to a decreased growth rate. Under extreme chronic metabolic stress (e.g. 27 °C), the effect is lethal. This new model needs to be tested but any large scale prediction of the combined impact of ocean warming and acidification would require to better understand the relative contribution of temperature, pH and any other modulating factors (e.g. food, Marsh and Manahan, 1999) on the metabolism of marine species (Kooijman, 2010).

4.2. Ecological consequences

A. lixula and its sister species *Arbacia punctulata* have been the object of intensive investigations in cell biology, biochemistry of fertilization and early development (e.g. Harding and Harding, 1952; Castagna et al., 1981; George et al., 1990; De Giorgi et al., 1991). Recently, renewed interest in this species arose mainly due to its ecological role, its unusually wide distribution area (from equatorial waters to temperate Mediterranean) and its warm-water affinity (Gianguzza et al., 2011; Wangensteen et al., 2012; Privitera et al., 2011; Agnetta et al., 2013).

A study on its phylogeography and population genetics corroborates the Stefanini (1911) hypothesis (backed by the lack of its fossil record in Mediterranean) that *A. lixula* is not a native species but a thermophilous colonizer, probably originated at tropical Atlantic region, which spread throughout the Mediterranean during the last interglacial period of the Pleistocene (Wangensteen et al., 2012). In this period, the minimum winter surface temperatures of the Mediterranean Sea had been warmer than 19 °C and this could have led larvae of the tropical Atlantic population of *A. lixula* to cross the Strait of Gibraltar and colonize the Northern Mediterranean. Furthermore, the authors suggested that the current population expansion of *A. lixula* in Mediterranean could be due to increased larval success driven by the ongoing climate warming.

It is well known that one direct consequence of warming is a simultaneous increase in the abundance of thermo-tolerant species and the disappearance or rarefaction of 'cold' stenothermal species (CIESM, 2008). From a study on climate warming and the range extension of thermophilic sea urchin species, a significant positive relationship between *C. rodgersii* density and increased ocean temperatures has been unequivocally proven in eastern Tasmania (Ling et al., 2008). Hart and Scheibling (1988) report evidence of an analogous temperature threshold mechanism for *Strongylocentrotus droebachiensis* along the Atlantic coast of Nova Scotia where sea urchin population booms and associated were correlated with a positive ocean temperature anomaly allowing optimal temperatures for larval development.

To date, few studies were focused on the impact of ocean acidification on *Arbacia* spp. development. The sub-Antarctic species *Arbacia dufresni* experienced a delay in development when raised at low pH with no effect on survival (Catarino et al., 2012b). Carr et al. (2006) showed that pH tolerance limits for the fertilization and embryological development (2 days) of *A. punctulata* were 6.9–8.8.

According to our data, it is likely that recent and future warming of the Western Mediterranean can result in an environment increasingly favourable for the reproduction and development of a thermotolerant species such as *A. lixula* (Privitera et al., 2011). We undoubtedly show that both fertilization and early larval development, using gamete from *A. lixula* adults acclimatized at 20 °C, were thermotolerant to 26° (+6 °C above sea surface temperature SST). Developmental rate was 1.7 times faster at 26 °C compared to 20 °C with no negative consequences on fertilization rate and survival. This also indicates that *A. lixula* may be in sub-optimal conditions in the Mediterranean and may then benefit from future ocean warming. Ocean acidification has a negative effect at the higher temperature (26 °C) showing that it may constrain the positive effect of warming. In the future ocean, larvae of the *A. lixula* may experience short-term exposure to these more challenging temperatures (>24 °C) with unknown consequences for their fitness. It is then difficult to make any prediction on the combined impact of ocean warming and acidification but it seems likely that on the short term, *A. lixula* can benefit from the positive effect of both warming and acidification on their larval growth rate and then experience a higher survival rate through decreased predation (Dupont et al., 2010c).

Calosi et al. (2013) showed that density of adult *A. lixula* was significantly greater than *P. lividus* in elevated PCO₂/low pH conditions. This pattern could be due to the relatively superior ability of *A. lixula* to regulate its extracellular fluid under elevated pCO₂ (Calosi et al., 2013).

If *A. lixula* increases its densities in the foreseeable future, it could have serious consequences for the Mediterranean ecosystem diversity and functioning. Many authors support the increasing evidence that *A. lixula*, less prone to predation than *P. lividus*, may establish a positive feedback which tends to stabilize and maintain the barren grounds in rocky littoral ecosystems (Bonaviri et al., 2011). Despite its ecological importance, no information was available on the impact of ocean acidification on *A. lixula*.

More research is needed to fully investigate the combined impacts of ocean acidification and warming on *A. lixula* including long-term exposure (including subsequent life-history stages, e.g. Dupont et al., 2012) allowing estimating acclimation and adaptation potential, the modulating role of ecological interactions and other environmental parameters (Kroeker et al., 2013). Future research should also aim at the investigation of the effects of multiple stressors on sea urchin recruitment and adult survival. This would allow obtaining the objective and completing the understanding of sea urchin population dynamic and potentially resultant effects on benthic community, in relation with global changes.

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References

- Agnetta, D., Bonaviri, C., Badalamenti, F., Scianna, C., Vizzini, S., Gianguzza, P., 2013. Functional traits of two co-occurring sea urchins across a barren/forest patch system. *Journal of Sea Research* 76, 170–177.
- Bonaviri, C., Pipitone, C., Gianguzza, P., Hereu, B., 2012. Micropredation on sea urchins as a potential stabilizing process for rocky reefs. *Journal of Sea Research* 73, 18–23.
- Bonaviri, C., Vega Fernández, T., Fanelli, G., Badalamenti, F., Gianguzza, P., 2011. Leading role of sea urchin *Arbacia lixula* in maintaining barren state in south-western Mediterranean. *Marine Biology* 158, 2505–2513.
- Byrne, M., Gonzalez-Bernat, M., Doo, S., Foo, S., Soars, N., Lamare, M., 2013. Effects of ocean warming and acidification on embryos and non-calcifying larvae of the invasive sea star *Patiriella regularis*. *Marine Ecology Progress Series* 473, 235–246.
- Byrne, M., 2011. Impact of ocean warming and ocean acidification on marine invertebrate life history stages. *Oceanography and Marine Biology An Annual Review* 49, 1–42. Edited by J. D. M. Gordon CRC Press.
- Byrne, M., Soars, N., Selvakumaraswamy, P., Dworjanyn, S.A., Davis, A.R., 2010a. Sea urchin fertilization in a warm, acidified and high pCO₂ ocean across a range of sperm densities. *Marine Environmental Research* 69, 234–239.
- Byrne, M., Soars, N.A., Ho, M.A., Wong, E., McElroy, D., Selvakumaraswamy, P., Dworjanyn, S.A., Davis, A.R., 2010b. Fertilization in a suite of coastal marine invertebrates from SE Australia is robust to near-future ocean warming and acidification. *Marine Biology* 157, 2061–2069.
- Byrne, M., Ho, M., Selvakumaraswamy, P., Nguyen, H.D., Dworjanyn, S.A., Davis, A.R., 2009. Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. *Proceedings of the Royal Society B* 276, 1883–1888.
- Caldeira, K., Wickett, M.E., 2003. Anthropogenic carbon and ocean pH. *Nature* 425, 365.
- Caldwell, G.S., Fitzner, S., Gillespie, C.S., Pickavance, G., Turnbull, E., Bentley, M.G., 2011. Ocean acidification takes sperm back in time. *Invertebrate Reproduction and Development* 55, 217–221.
- Calosi, P., Rastrick, S.P.S., Graziano, M., Thomas, S.C., Baggini, C., Carter, H.A., Hall-Spencer, J.M., Milazzo, M., Spicer, J.L., 2013. Distribution of sea urchins living near shallow water CO₂ vents is dependent upon species acid–base and ion-regulatory abilities. *Marine Pollution Bulletin*, 1–15. <http://dx.doi.org/10.1016/j.marpolbul.2012.11.040>.
- Carr, R.S., Biedenbach, J.M., Nipper, M., 2006. Influence of potentially confounding factors on sea urchin porewater toxicity tests. *Archives of Environmental Contamination and Toxicology* 51, 573–579.
- Castagna, C., Sinatra, F., Scalia, M., Capodicasa, V., 1981. Observations of the effect of zinc on the gametes and various development phases of *Arbacia lixula*. *Marine Biology* 64, 285–289.
- Catarino, A.I., Bauwens, M., Dubois, P., 2012a. Acid–base balance and metabolic response of the sea urchin *Paracentrotus lividus* to different seawater pH and temperatures. *Environmental Science and Pollution Research*. <http://dx.doi.org/10.1007/s11356-012-0743-1>.
- Catarino, A.I., De Ridder, C., Gonzalez, M., Gallardo, P., Dubois, P., 2012b. Sea urchin *Arbacia dufruesnei* (Blainville 1825) larvae response to ocean acidification. *Polar Biology* 35, 455–461.
- Cerrano, C., Bavestrello, G., Bianchi, C.N., Cattaneo-Vietti, R., Bava, S., Morganti, C., Morri, C., Picco, P., Sara, G., Schiaparelli, S., Siccardi, A., Sponga, F., Siccardi, A., 2000. A catastrophic mass-mortality episode of gorgonians and other organisms in the Ligurian Sea (North-western Mediterranean), summer 1999. *Ecology Letters* 3, 284–293.
- CIEM, 2008. Climate warming and related changes in Mediterranean marine biota, N° 35. In: Briand, F. (Ed.), CIEM Workshop Monographs, Monaco.
- De Giorgi, C., De Luca, F., Saccone, C., 1991. Mitochondrial DNA in the sea urchin *Arbacia lixula*: nucleotide sequence differences between two polymorphic molecules indicate asymmetry of mutations. *Gene* 103, 249–252.
- Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Research* 34, 1733–1743.
- Duarte, C.M., 2007. Marine ecology warms up to theory. *Trends in Ecology and Evolution* 22, 331–333.
- Dupont, S., Havenhand, J., Thorndyke, W., Peck, L., Thorndyke, M., 2008. Near-future level of CO₂-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. *Marine Ecology Progress Series* 373, 285–294.
- Dupont, S., Thorndyke, M., 2013. Direct impacts of near-future ocean acidification on sea urchins. In: *Climate Change Perspectives from the Atlantic: Past, Present and Future*.
- Dupont, S., Ortega-Martinez, O., Thorndyke, M., 2010a. Impact of near-future ocean acidification on echinoderms. *Ecotoxicology* 19, 449–462.
- Dupont, S., Lundve, B., Thorndyke, M., 2010b. Near future ocean acidification increases growth rate of the lecithotrophic larvae and juveniles of the sea star *Crossaster papposus*. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution* 314 B, 382–389.
- Dupont, S., Dorey, N., Thorndyke, M., 2010c. What meta-analysis can tell us on vulnerability of marine biodiversity to ocean acidification? *Estuarine, Coastal and Shelf Science* 89, 182–185.
- Ericson, J.A., Ho, M.A., Miskelly, A., King, C.K., Virtue, P., Tilbrook, B., Byrne, M., 2012. Combined effects of two ocean change stressors, warming and acidification, on fertilization and early development of the Antarctic echinoid *Sterechinus neumayeri*. *Polar Biology* 35, 1027–1034.
- Ericson, J.A., Lamare, M.D., Morley, S.A., Barker, M.F., 2010. The response of two ecologically important Antarctic invertebrates (*Sterechinus neumayeri* and *Parborlasiella corrugatus*) to reduced seawater pH: effects on fertilisation and embryonic development. *Marine Biology* 157, 2689–2702.
- Fabry, V.J., Seibel, B.A., Feely, R.A., Orr, J.C., 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science* 65, 414–432.
- Fenaux, L., 1968. Maturation des gonades et cycle saisonnier des larves chez *A. lixula*, *Plividus* et *P. microtuberculatus* à Villefranche- Sur-Mer. *Vie Milieu Serie A* 19, 1–52.
- Foo, S.A., Dworjanyn, S.A., Poore, A.G.B., Byrne, M., 2012. Adaptive capacity of the habitat modifying sea urchin *Centrostephanus rodgersii* to ocean warming and ocean acidification: performance of early embryos. *PLoS ONE* 7, e42497.
- Francoeur, P., Boudouresque, C.F., Harmelin, J.G., Harmelin-Vivien, M.L., Quignard, J.P., 1994. Are the Mediterranean waters becoming warmer? information from biological indicators. *Marine Pollution Bulletin* 28, 523–526.
- Garrabou, J., Coma, R., Bensoussan, N., Bally, M., Chevaldonné, P., Cigliano, M., Diaz, D., Harmelin, J.G., Gambi, M.C., Kersting, D.K., Ledoux, J.B., Lejeune, C., Linares, C., Marschal, C., Pérez, T., Ribes, M., Romano, J.C., Serrano, E., Teixido, N., Torrents, O., Zabala, M., Zuberer, F., Cerrano, C., 2009. Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. *Global Change Biology* 15, 1090–1103.
- George, S.B., Cellario, C., Fenaux, L., 1990. Population differences in egg quality of *Arbacia lixula* (Echinodermata: Echinoidea): proximate composition of eggs and larval development. *Journal of Experimental Marine Biology and Ecology* 141, 107–118.
- Gianguzza, P., Agnetta, D., Bonaviri, C., Di Trapani, F., Visconti, G., Gianguzza, F., Riggio, S., 2011. The rise of thermophilic sea urchins and the expansion of barren grounds in the Mediterranean Sea. *Chemistry and Ecology* 27, 129–134.
- Gianguzza, P., Bonaviri, C., 2013. *Arbacia*. *Developments in Aquaculture and Fisheries Science* 38, 275–283.
- Gonzalez-Bernat, M.J., Lamare, M., Barker, M., 2013. Effects of reduced seawater pH on fertilisation, embryogenesis and larval development in the Antarctic seastar *Odontaster validus*. *Polar Biology* 36, 235–247.
- Gonzalez-Bernat, M.J., Lamare, M., Uthicke, S., Byrne, M., 2012. Fertilization, embryogenesis and larval development in the tropical intertidal sand dollar *Arachnoides placenta* in response to reduced seawater pH. *Marine Biology*. <http://dx.doi.org/10.1007/s00227-012-2034-2>.
- Guidetti, P., Dulčić, J., 2007. Relationship among predatory fish, sea urchins and barrens in Mediterranean rocky reefs across a latitudinal gradient. *Marine Environmental Research* 63, 168–184.
- Harding, C.V., Harding, D., 1952. Cross fertilization with the sperm of *Arbacia lixula*. *Experimental Cell Research* 3, 475–484.
- Hart, M.W., Scheibling, R.E., 1988. Heat waves, baby booms, and the destruction of kelp beds by sea urchins. *Marine Biology* 99, 167–176.
- Havenhand, J.N., Butler, F.R., Thorndyke, M.C., Williamson, J.E., 2008. Near-future levels of ocean acidification reduce fertilization success in a sea urchin. *Current Biology* 18, 651–652.
- Hereu, B., Linares, C., Sala, E., Garrabou, J., Garcia-Rubies, A., Diaz, D., Zabala, M., 2012. Multiple processes regulate long-term population dynamics of sea urchins on Mediterranean rocky reefs. *PLoS ONE* 7 art. no. e36901.
- IPCC (Intergovernmental Panel on Climate Change), 2007. *The Fourth Assessment Report of the IPCC*. Cambridge University Press, Cambridge, UK.
- Kooijman, S.A.L.M., 2010. *Dynamic Energy Budget Theory for Metabolic Organization*. Cambridge University Press, Cambridge, UK.
- Kroeker, K.J., Micheli, F., Gambi, M.C., 2013. Ocean acidification causes ecosystem shifts via altered competitive interactions. *Nature Climate Change* 3, 156–159.
- Kronström, J., Dupont, S., Malfet, J., Thorndyke, M., Holmgren, S., 2007. Serotonin and nitric oxide interaction in the control of bioluminescence in northern krill, *Meganyctiphanes norvegica* (M. Sars). *Journal of Experimental Biology* 209, 3179–3187.
- Kurihara, H., Shirayama, Y., 2004. Effects of increased atmospheric CO₂ on sea urchin early development. *Marine Ecology Progress Series* 274, 161–169.
- Kurihara, H., Shimode, S., Shirayama, Y., 2004. Sub-lethal effects of elevated concentration of CO₂ on planktonic copepods and sea urchins. *Journal of Oceanography* 60, 743–750.
- Lessios, H.A., Lockhart, S., Collin, R., Sotil, G., Sanchez-Jerez, P., Zigler, K.S., Perez, A.F., Garrido, M.J., Geyer, L.B., Bernardi, G., Vacquier, V.D., Haroun, K., Kessing, B.D., 2012. Phylogeography and bindin evolution in *Arbacia*, a sea urchin genus with an unusual distribution. *Molecular Ecology* 21, 130–144.
- Lewis, E., Wallace, D.W.R., 1998. CO₂SYS—Program Developed for the CO₂ System Calculations. Carbon Dioxide Inf Anal Center Report ORNL/CDIAC-105.
- Ling, S.D., Johnson, C.R., Frusher, S., King, C.K., 2008. Reproductive potential of a marine ecosystem engineer at the edge of a newly expanded range. *Global Change Biology* 14, 907–915.

- Marsh, A.G., Manahan, D.T., 1999. A method for accurate measurements of the respiration rates of marine invertebrate embryos and larvae. *Marine Ecology Progress Series* 184, 1–10.
- Martin, S., Richier, S., Pedrotti, M.L., Dupont, S., Castejon, C., Gerakis, Y., Kerros, M.E., Oberhänsli, F., Teysié, J.L., Jeffree, R., Gattuso, J.P., 2011. Early development and molecular plasticity in the Mediterranean sea urchin *Paracentrotus lividus* exposed to CO₂-driven acidification. *Journal of Experimental Biology* 214, 1357–1368.
- Mehrbach, C., Culbertson, C.H., Hawley, J.E., Pytkowicz, R.M., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography* 18, 897–907.
- Moulin, L., Catarino, A.I., Claessens, T., Dubois, P., 2011. Effects of seawater acidification on early development of the intertidal sea urchin *Paracentrotus lividus* (Lamarck 1816). *Marine Pollution Bulletin* 62, 48–54.
- Nguyen, H.D., Doo, S.S., Soars, N.A., Byrne, M., 2012. Noncalcifying larvae in a changing ocean: warming, not acidification/hypercapnia, is the dominant stressor on development of the sea star *Meridiastra calcar*. *Global Change Biology* 18, 2466–2476.
- Peck, L.S., Prothero-Thomas, E., 2002. Temperature effects on the metabolism of larvae of the Antarctic starfish *Odontaster validus*, using a novel micro-respirometry method. *Marine Biology* 141, 271–276.
- Pedrotti, M.L., 1993. Spatial and temporal distribution and recruitment of echinoderm larvae in the Ligurian Sea. *Journal Marine Biological Association UK* 73, 513–530.
- Podolsky, R., Emler, R.B., 1993. Partitioning the effects of temperature and viscosity on swimming and water movement by sand dollar larvae (*Dendraster excentricus*). *Journal of Experimental Biology* 176, 207–221.
- Pörtner, H.O., Farrell, A.P., 2008. Physiology and climate change. *Science* 322, 690–692.
- Privitera, D., Noli, M., Falugi, C., Chiantore, M., 2011. Benthic assemblages and temperature effects on *Paracentrotus lividus* and *Arbacia lixula* larvae and settlement. *Journal of Experimental Marine Biology and Ecology* 407, 6–11.
- Reuter, K.E., Lotterhos, K.E., Crim, R.N., Thompson, C.A., Harley, C.D.G., 2011. Elevated pCO₂ increases sperm limitation and risk of polyspermy in the red sea urchin *Strongylocentrotus franciscanus*. *Global Change Biology* 17, 163–171.
- Rivarolo, P., Messa, R., Massolo, S., Frache, R., 2010. Distributions of carbonate properties along the water column in the Mediterranean Sea: spatial and temporal variations. *Marine Chemistry* 121, 236–245.
- Russell, B.D., Harley, C.D.G., Wernberg, T., Mieszkowska, N., Widdicombe, S., Hall-Spencer, J.M., Connell, S.D., 2012. Predicting ecosystem shifts requires new approaches that integrate the effects of climate change across entire systems. *Biology Letters* 8, 164–166.
- Sala, E., Boudouresque, C.F., Harmelin-Vivien, M., 1998. Fishing, trophic cascades, and the structure of algal assemblages: evaluation of an old but untested paradigm. *Oikos* 82, 425–439.
- Sarà, G., Milanese, M., Prusina, I., Sarà, A., Angel, D.L., Glamuzina, B., Nitzan, T., Freeman, S., Rinaldi, A., Palmeri, V., Montalto, V., Lo Martire, M., Gianguzza, P., Arizza, V., Lo Brutto, S., De Pirro, M., Helmuth, B., Murray, J., De Cantis, S., Williams, G.A., 2012. The impact of climate change on Mediterranean intertidal communities: losses in coastal ecosystem integrity and services. *Regional Environmental Change*. <http://dx.doi.org/10.1007/s10113-012-0360-z> (in press).
- Sarà, G., Kearney, M., Helmuth, B., 2011. Combining heat-transfer and energy budget models to predict local and geographic patterns of mortality in Mediterranean intertidal mussels. *Chemistry and Ecology* 27, 135–145.
- Schlegel, P., Havenhand, J.N., Gillings, M.R., Williamson, J.E., 2012. Individual variability in reproductive success determines winners and losers under ocean acidification: a case study with sea urchins. *PLoS ONE* 7 art. no. e53118.
- Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52, 591–611.
- Sheppard Brenndand, H., Soars, N., Dworjanyn, S.A., Davis, A.R., Byrne, M., 2010. Impact of ocean warming and ocean acidification on larval development and calcification in the sea urchin *Tripneustes gratilla*. *PLoS ONE* 5, e11372.
- Sokolova, I.M., Frederich, M., Bagwe, R., Lannig, G., Sukhotin, A.A., 2012. Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Marine Environmental Research* 79, 1–15.
- Stefanini, G., 1911. Di alcune *Arbacia* fossili. *Rivista Italiana di Paleontologia* 17, 51–52.
- Stumpp, M., Hu, M.Y., Melzner, F., Gutowska, M.A., Dorey, N., Himmerkus, N., Holtmann, W.C., Dupont, S.T., Thorndyke, M.C., Bleich, M., 2012. Towards improved socio-economic assessments of ocean acidification's impacts. *Proceedings of the National Academy of Sciences of the United States of America* 109, 18192–18197.
- Stumpp, M., Wren, J., Melzner, Frank, Thorndyke, Mike, Dupont, S., 2011. CO₂ induced seawater acidification impacts sea urchin larval development I: elevated metabolic rates decrease scope for growth and induce developmental delay. *Comparative Biochemistry and Physiology A-molecular & Integrative Physiology* 160, 331–340.
- Wangensteen, O.S., Turon, X., García-Cisneros, A., Recasens, M., Romero, J., Palacín, C., 2011. A wolf in sheep's clothing: carnivory in dominant sea urchins in the Mediterranean. *Marine Ecology Progress Series* 441, 117–128.
- Wangensteen, O.S., Turon, X., Pérez-Portela, R., Palacín, C., 2012. Natural or Naturalized? Phylogeography suggests that the abundant sea urchin *Arbacia lixula* is a recent colonizer of the Mediterranean. *PLoS ONE* 7 (9), e45067. <http://dx.doi.org/10.1371/journal.pone.0045067>.