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Responses of sea urchin larvae to field and laboratory acidification

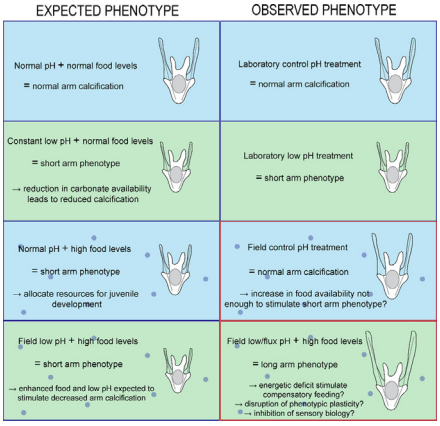
Shawna A. Foo [a,b,*], David A. Koweek [b], Marco Munari [c], Maria Cristina Gambi [c], Maria Byrne [d], Ken Caldeira [b]

[a] Center for Global Discovery and Conservation Science, Arizona State University, Tempe, AZ 85287, USA
[b] Department of Global Ecology, Carnegie Institution for Science, 260 Panama Street, Stanford, CA 94305, USA
[c] Department of Integrative Marine Ecology, Ischia Marine Centre, Stazione Zoologica Anton Dohrn, Punta San Pietro, 80077, Ischia(Naples), Italy
[d] School of Medical Sciences and School of Life and Environmental Sciences, The University of Sydney, Sydney, New South Wales 2006, Australia

HIGHLIGHTS

- We deploy identical populations of sea urchin embryos in laboratory and field ocean acidification scenarios.
- Acidification had opposite effects on larval growth in the laboratory and in the field.
- Results of ocean acidification laboratory studies may not necessarily scale to field conditions.

GRAPHICAL ABSTRACT



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ABSTRACT

Understanding the extent to which laboratory findings of low pH on marine organisms can be extrapolated to the natural environment is key toward making better projections about the impacts of global change on marine ecosystems. We simultaneously exposed larvae of the sea urchin *Arbacia lixula* to ocean acidification in laboratory and natural CO_2 vents and assessed the arm growth response as a proxy of net calcification. Populations of embryos were simultaneously placed at both control and volcanic CO_2 vent sites in Ischia (Italy), with a parallel group maintained in the laboratory in control and low pH treatments corresponding to the mean pH levels of the field sites. As expected, larvae grown at constant low pH (pH_T 7.8) in the laboratory exhibited reduced arm growth, but counter to expectations, the larvae that developed at the low pH vent site (pH_T 7.33–7.99) had the longest arms. The larvae at the control field site (pH_T 7.87–7.99) grew at a similar rate to laboratory controls. Salinity, temperature, oxygen and flow regimes were comparable between control and vent sites; however, chlorophyll *a* levels and particulate organic carbon were higher at the vent site than at the control field site. This increased food availability may have modulated the effects of low pH, creating an opposite calcification response in the laboratory from that in the field. Divergent responses of the same larval populations developing in laboratory and field environments show the importance of considering larval phenotypic plasticity and the complex interactions among decreased pH, food availability and larval responses.

* Corresponding author at: Center for Global Discovery and Conservation Science, Arizona State University, Tempe, Arizona USA 85287.
E-mail address: sfoo@asu.edu (S.A. Foo).

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1. Introduction

Oceanic uptake of 30% of anthropogenic CO_2 emissions has altered carbonate chemistry, resulting in ocean acidification (OA) (Caldeira and Wickett, 2005). Ocean acidification changes the equilibrium of the seawater carbonate system, decreasing the thermodynamic tendency to precipitate carbonate minerals, and exposing organisms to hypercapnia, a metabolic stressor (Pörtner, 2010). These changes can impair the ability of calcifying marine invertebrates to build their skeletons as shown under experimental (laboratory) (*e.g.* Przeslawski et al., 2015; Munari et al., 2016) and natural (CO_2 seeps) acidification (Fabricius et al., 2011; Foo et al., 2018).

Most OA studies on marine calcifiers have been conducted in a laboratory setting, typically involving stable pH conditions. These studies show the overall negative effect of acidification with impaired development and calcification (Byrne et al., 2013; Kroeker et al., 2013a; Przeslawski et al., 2015; Byrne and Fitzer, 2019; Byrne and Hernández, 2020). Whether these findings would occur in natural environments is not known because it is difficult to incorporate the range of environmental factors present in nature and their inherent variability in a laboratory setting (Harvey et al., 2013; Ban et al., 2014; Nagelkerken and Munday, 2016). To understand the response of species to OA, an understanding of organisms' responses to natural variation in environmental conditions is needed (Hurd et al., 2011; Small et al., 2015).

The difference between laboratory and field studies has been examined by utilising several natural systems. A study on the response of the rotifer *Keratella taurocephala* to decreased pH in the laboratory and an acidified lake (pH 4.7–5.6) found that the effects of pH and food were not as strong in the lake due to a reduction in predator abundance (Gonzalez and Frost, 1994). In another acidified lake scenario, multiple stressors such as shifts in microbial processes and changes in water clarity, arose from the single stress of acidification indicating that various mechanisms could affect organismal changes (Frost et al., 1999)

CO_2 vents, another natural system, have been used as proxies to investigate the impacts of OA. These systems incorporate a range of environmental factors including planktonic food webs, productivity, water flow, and species interactions that are also affected by changes in pH (Kroeker et al., 2011; Connell et al., 2013). Fluctuating pH levels are characteristic of other natural oceanic systems such as tide pools, coral reefs and areas of natural upwelling; however, the temporal variability of vent site carbonate chemistry is more pronounced (Hofmann et al., 2011), where pH can vary by 0.1 units within an hour (Foo et al., 2018).

At vent sites, productivity can be higher than in surrounding water due to the enhancement of phytoplankton growth from high concentrations of CO_2 that increase the supply of inorganic carbon for photosynthesis (Lamare et al., 2016; Uthicke et al., 2016; Doubleday et al., 2019). Algal biomass increases along the natural low pH gradients characteristic of vent sites, where elevated algal productivity is an important food source for marine invertebrates (Uthicke et al., 2016). For some species, increased food availability at vent sites outweighs the negative effects of acidification (Connell et al., 2017; Doubleday et al., 2019).

Sea urchins calcify in their planktonic and benthic life stages and are used as a model taxon to investigate the effects of OA in laboratory (Byrne et al., 2013; Przeslawski et al., 2015) and in CO_2 vent studies (Kroeker et al., 2013b; Foo et al., 2018). These invertebrates are ecologically important grazers that mediate ecosystem transitions in temperate kelp (Lawrence, 2013; Ling et al., 2018) and tropical coral ecosystems (Sandin and McNamara, 2012). Sea urchin larvae produce fragile skeletons and a meta-analysis of data from 15 species across world latitudes show that laboratory OA ($pCO_2 \geq 1000$ μatm/pH ≤ 7.6) had a stunting effect on larval growth largely driven by hypercapnia and regardless of phylogeny, whereas progeny of sea urchins resident in fluctuating pH environments were more resilient (Byrne et al., 2013; Wong et al., 2018).

Arbacia lixula, the sea urchin used in this study, is a particularly important keystone ecosystem engineer in the Mediterranean due to its ability in transforming macroalgal communities into barren grounds due to grazing (Gianguzza, 2020). As a thermophilous species, warming of the Mediterranean has supported an increase in abundance of this sea urchin where reproduction and larval development are enhanced by high temperature and modulated by pH and food availability (Gianguzza et al., 2011; Wangensteen et al., 2013; Gianguzza, 2020). It is therefore of utmost importance to determine the vulnerability of this species to OA in natural scenarios to increase our understanding of the species resilience and capabilities in the context of climate change.

Most laboratory studies of the calcification response of sea urchin larvae to OA have used constant pH treatments, although recent studies have explored the impacts of fluctuating pH, emulating what might occur in a future ocean. Responses of larvae to fluctuating pH are mixed (review Kapsenberg and Cyronak, 2019), where developmental rates have either been enhanced (García et al., 2018) or suppressed (Peeters, 2013). Other calcifying organisms show that growth correlates with mean pH exposure rather than pH variability (Kapsenberg et al., 2018) or that pH variability exacerbates the negative effects of low pH (Johnson et al., 2019).

Organismal responses to high CO_2 exposure in the field are more complicated. Sea urchin larvae reared at a CO_2 vent site in Papua New Guinea were smaller than those reared at the control field site (Lamare et al., 2016). Decreased larval calcification at vent sites may be due to the direct effects of CO_2, but interpreting these findings in a natural setting can be difficult because of phenotypically plastic arm growth of sea urchin larvae in response to the nutritive environment. The larvae of many sea urchins grow longer arms in low food conditions as a means to facilitate capture of scarce food and shorter arms in higher food conditions to allocate more resources to juvenile development and reduce the time to metamorphosis (Pedrotti and Fenaux, 1992; Strathmann et al., 1992; Soars et al., 2009; Kalachev et al., 2018). Differences in arm growth are also influenced by genetic variability as shown in the response of different sea urchin families to OA (Foo and Byrne, 2016).

We used the volcanic vent areas of Ischia, Italy to investigate the impacts of field (vent CO_2 release) and experimental (laboratory CO_2 dosing) acidification on the calcification response of larvae of the Mediterranean sea urchin *Arbacia lixula*, a sea urchin that also resides at the vent sites. In conjunction with larval deployments, we characterized the environmental factors (*e.g.* pH, oxygen, salinity, chl *a*) that may influence sea urchin growth and calcification. As parental source can influence larval growth, we performed three separate fertilisations using multiple males and females to examine effects across multiple independent populations. We documented the growth response of larvae to OA in field and laboratory experiments, and hypothesized that they would show reduced calcification under acidification in the laboratory and at the vent site compared with ambient conditions. Our field results challenged our hypothesis, where longer arm length was observed at the high CO_2 vent site.

2. Methods

2.1. Study species and collection site

Arbacia lixula were collected in September 2017 during their spawning season (adults are mature from May–October (Wangensteen et al., 2013)) at S. Pietro, Ischia, Italy (40° 44′ 46.70″ N; 13° 56′ 40.95″ E), approximately 4 km from the Castello vent system where the experiments were undertaken (see below). The seawater at S. Pietro has a mean pH_T 8.001 (pH on the total hydrogen ion scale at *in situ* temperature) (Foo et al., 2018). The urchins were kept in cool boxes, and then transferred into a large 200 L holding tank with ambient flow-through conditions at the Villa Dohrn Benthic Ecology Center in Ischia (Stazione Zoologica Anton Dohrn, Naples, Italy).

2.2. Spawning and fertilization procedure

Arbacia lixula were spawned within five days of collection by injection of 2–4 mL of 0.5 M KCl. Eggs were collected into beakers (500 mL) of 0.45 μm filtered seawater (FSW) at ambient pH. Sperm was collected dry and kept on ice until use.

Three independent fertilizations were performed to create three different populations of embryos (Fig. 1). For each fertilization, five males and five females were spawned. Therefore, each independent group (population) was a mixture of sibling and non-sibling embryos depending on the interaction at fertilization between the gametes of the ten parents used. Equal amounts of eggs from each female were mixed and placed in a 2.5 L beaker filled with FSW. Egg density was determined in counts of 100 mL aliquots. Equal parts of sperm from the males were mixed and activated in seawater just prior to fertilization. Hemocytometer counts of sperm samples diluted with experimental FSW were used to determine the amount required to achieve a final sperm concentration of 10^4 sperm/mL. After 10 min, fertilization success was checked and in all cases was >95% for each trial. The embryos were rinsed to remove excess sperm and were then transferred to experimental 'larval homes' within 1 h.

2.3. Simultaneous laboratory and field experiments

The three populations of sea urchin embryos were simultaneously exposed to control and low pH flow-through conditions in the laboratory, as well as to control and low pH conditions in the field, for a total of four treatments (Fig. 1). The embryos were transferred into 200 mL 'larval homes' at a concentration of 10 embryos per mL. Each larval home consisted of a 14 × 3.5 cm pipe with 64 μm mesh caps on both ends to allow water exchange through the larval homes (Fig. 2). Larval homes were tested prior to the experiment with rhodamine dye in a large, flow-through bath to ensure that the mesh allowed free flow through the container. Larval homes have also been used in previous experiments successfully for sea urchin larvae and adult invertebrates (Frieder, 2013; Calosi et al., 2013; Ricevuto et al., 2015; Turner et al., 2016). Each population was split across two experimental pH levels in the laboratory and in the field, with six replicates per condition, resulting in 24 larval homes per population; a total of 72 larval homes for the experiment. Larval homes were divided equally and placed into each experimental tank so that all three populations were simultaneously exposed to all treatments.

In the field, larval homes were deployed at two sites: a control site (pH_T 7.95 ± 0.003, mean ± SE, Fig. 3; Table 1) and a CO_2 vent site (pH_T 7.80 ± 0.011, mean ± SE, Fig. 3; Table 1) at *in situ* temperature. The low pH, vent site of 3 m depth was selected on the north side of the pH gradient (40° 43′ 55.83″ N; 13° 57′ 52.14″ E); a zone previously defined as low pH or N2 station in previous studies (see review, Foo et al., 2018). The control pH station was also located on the north side of the Castello Aragonese, approximately 150 m from the vent site (40° 43′ 56.88″ N; 13° 57′ 56.56″ E) and at comparable depth (3.5 m). Larval homes were attached to a mooring (25 kg blocks) with cable ties at 2 m below the sea surface (approx. 1.5 m from the bottom). Larval homes across populations were arranged randomly to the mooring.

In parallel with the field set up, larval homes were deployed into two treatments in the laboratory. Seawater was supplied to outdoor experimental tanks from a flow-through seawater system at two pH_T levels (mean ± SE at *in situ* temperature): control pH 8.01 ± 0.002 and low pH 7.80 ± 0.002, Fig. 1; Table 1. Experimental pH for the low pH treatment was controlled *via* a mixed CO_2 supply where a pH controller (Apex Systems, Neptune) regulated the amount of CO_2 gas supplied into the airline. The required amount of air and CO_2 was bubbled through ceramic diffusers into an 80 L header tank, controlled by an automatic CO_2 injection and pH-controlled feedback system (Apex Systems, Neptune). The header tank flowed into three 20 L experimental tanks (60 mL/min), which maintained even mixing of water. Water

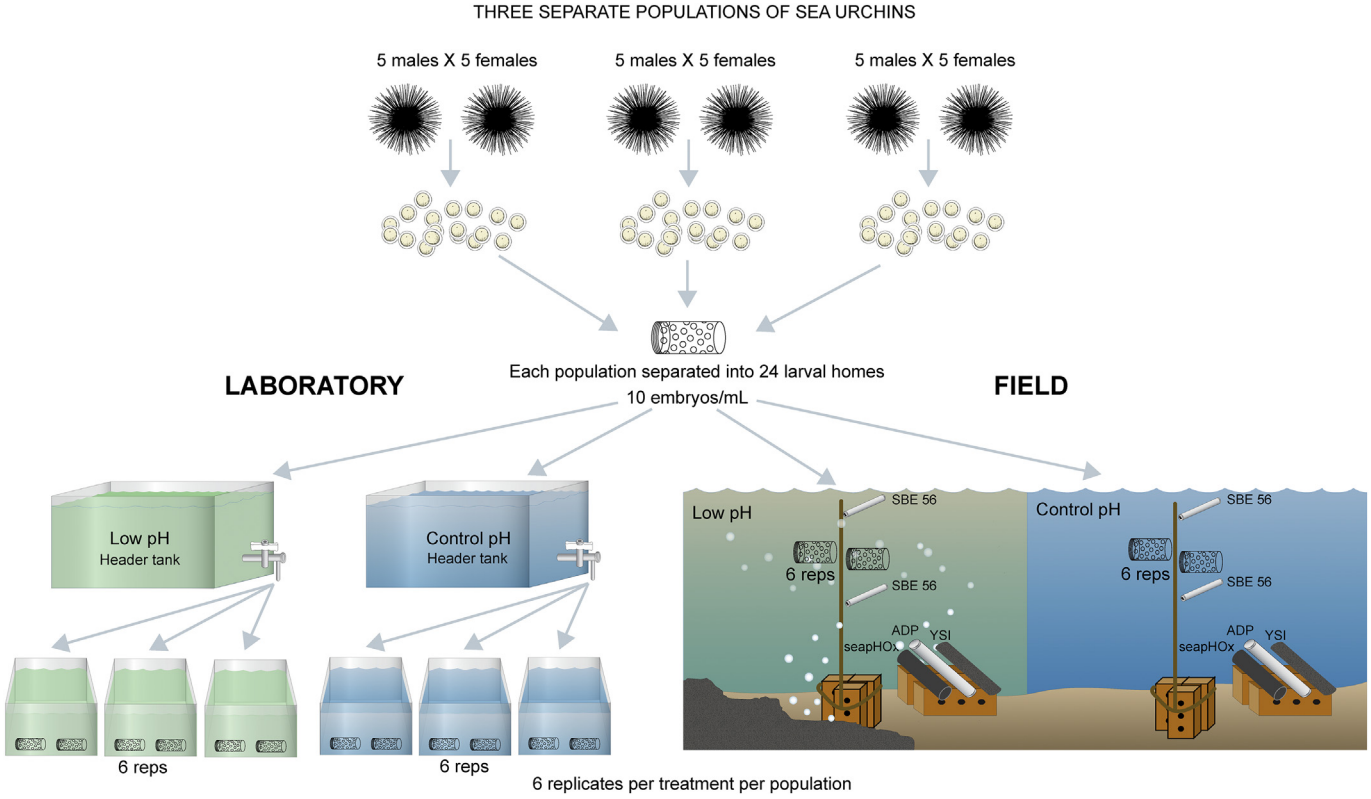

Fig. 1. To simultaneously compare the response of the development of *A. lixula* to laboratory and field acidification, three different populations of embryos were created in three independent fertilizations (see Methods section). Embryos in larval homes (Fig. 2) were exposed to control and low pH in laboratory flow-through tanks and in the field to control and low pH vent sites, resulting in a total of four treatments.

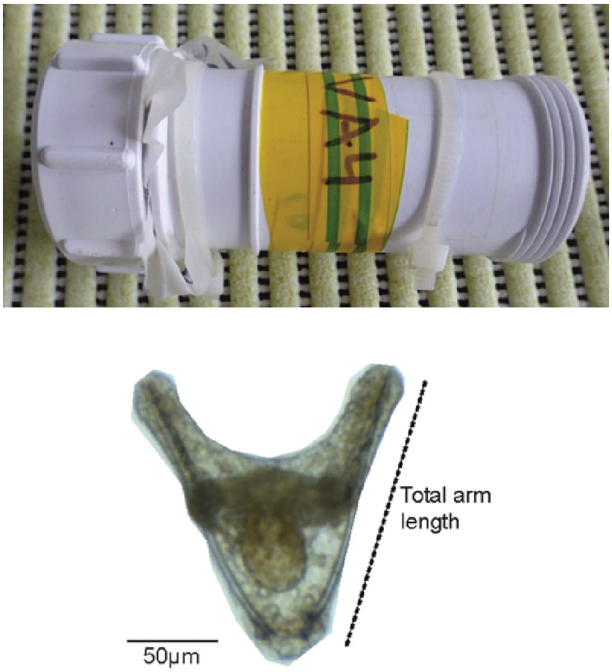

Fig. 2. Each larval home consisted of a 14 cm long and 3.5 cm diameter pipe coupled with a cap on either end, which holds 64 µm mesh in place. The top panel displays a larval home with one end capped, and the other end uncapped to allow the transfer of larvae into the home. The bottom panel displays a 24 h old *Arbacia lixula* larva with the measurement of total arm length indicated.

temperature was monitored in each tank with temperature loggers (SBE 56, Sea-Bird Electronics, Inc.). The control treatment had the same set up with one header tank flowing to three experimental tanks. pH was also manually checked using a pH meter (Mettler Toledo SevenGo), which was calibrated frequently using NIST buffers pH 4.0, 7.0 and 10.0.

After transfer of embryos to the larval homes, the larval homes for field deployment were placed inside coolers of control seawater and transported by boat to the field site close by (4 km). The larval homes were attached to the moorings at the vent site first and the control site 25 min after. The field and laboratory teams coordinated so that laboratory larval homes were deployed at similar times. Thus, the transfer into the laboratory and field experiments was completed within 2 h from fertilization on September 12, 2017. The experiment was run simultaneously for the three populations and thus they all experienced the same experimental conditions. The embryos were left to develop to the feeding larval stage, when the posterolateral arms are well-developed and the gut has just completed (Fig. 2). This developmental stage is commensurate with that used in other studies of echinoplutei (Byrne et al., 2013). In *A. lixula,* this stage is reached by 24 h (Fig. 2), earlier than in other sea urchin species (George, 1990; Hadfield and Strathmann, 1996). Thus, our deployments were collected after 24 h, particularly important as we were interested in assessing the initial calcification response with the expectation of stunted arm growth, as exemplified by multiple studies (Byrne et al., 2013). This also facilitated retention of sufficient numbers of larvae measurements as we noted loss over time in a previous pilot study (in 2016) where the deployments were longer in duration. Larvae were filtered out of the larval homes and collected into 2 mL Eppendorf tubes and preserved in 2.5% seawater buffered paraformaldehyde overnight. The next day they were transferred into 1× phosphate-buffered saline solution. All larvae recovered from the experiment were photographed. Total arm length (Fig. 2) for 30 larvae from each replicate were measured using Image J (U. S. National Institutes of Health), resulting in a total of 2160 separate measurements.

2.4. Field site characterization

Autonomous sensors were deployed at both field sites for nine days (from seven days before until one day after the completion of the experiment) to document biogeochemical and hydrological parameters (Figs. 1, S2). SeapHOxes were deployed at the vent and control sites to record pH, temperature, salinity and oxygen (Bresnahan et al., 2014) with measurements taken every 10 min at the vent site and 12 min at the control site. YSI data sondes (Model no. 6600) recorded chl *a* concentration every minute at the vent site and every 2 min at the control site. Acoustic Doppler profilers (Nortek Aquadopp Profile 1 MHz, standard resolution), measured three-dimensional water velocities at both the control and vent sites. Sensors were intercalibrated before and after the field deployment in a large common bath, during which water samples were collected to calibrate the pH sensor (Bresnahan et al., 2014). We also conducted CTD hydrocasts (CastAway, Xylem Inc.) at each field station at the start and conclusion of the experiment to characterize the vertical water column structure (Fig. S1). We characterized the field site over a week, but focus on the 24 h that the larvae were *in situ* in the presentation of the results.

2.5. Seawater chemistry

Discrete water samples were collected at four time points in the field: the start (9th September 2017) and end (15th September 2017) of sensor deployment, and at the start (12th September 2017) and end (13th September 2017) of larval home deployment (N = 4 per treatment). Discrete water samples were collected at two time points in the laboratory: at the start and end of larval home deployment, in each individual tank (N = 6 per treatment). We used 5 L Niskin bottles to sample water at the depth of the larval homes at the field sites, and directly collected water samples from each lab tank (*i.e.* individual tanks, not the header tank) using 500 mL beakers. The water samples were used for chl *a* (500 mL), POC (1 L), total alkalinity (TA) (500 mL), and spectrophotometric pH_T (500 mL) analyses.

For chl *a* concentrations, the water samples were filtered onto 47 mm GF/F Whatman filters and extracted using 10 mL of 90% acetone (HPLC grade). These samples were kept dark and cool at 4 °C for 24 h before analysing by spectrophotometry. Chlorophyll *a* levels were calculated using the equation: chl $a = (11.85 \times A_{664}) - (0.08 \times A_{630})$ (Jeffrey and Humphrey, 1975).

For POC, the water samples were filtered through pre-combusted (450 °C for 4.5 h) filters (25 mm, 0.7 µm Whatman GF/F). Filters were rinsed immediately after filtration with 0.2 N HCL to remove calcium carbonate and dried in a desiccator. Filters were stored in separate petri dishes and later analysed at the Stable Isotope Laboratory at Stanford University with a Carlo Erba NA1500 Series 2 elemental analyser coupled to a Finnigan Delta + mass spectrometer with a ConFloII open split interface. Eleven NIST RM 8573 (USGS-40) standards were used to calibrate the analyser.

For TA and spectrophotometric pH, water was preserved by addition of 300 µL saturated mercuric chloride following (Dickson et al., 2007). Samples were analysed within one month of collection at Bodega Marine Laboratory (Bodega Bay, CA, USA). Seawater pH_T was measured spectrophotometrically using the pH-sensitive dye m-cresol purple (Zeebe and Wolf-Gladrow, 2001). Each water sample was measured in triplicate with immediate duplicate analyses of samples yielding an instrumental precision for triplicates of 0.0027 pH_T units. Total alkalinity was determined using potentiometric acid titration using Certified Reference Materials (CRM) provided by A. Dickson (Scripps Institution of Oceanography; Dickson et al., 2007). Sample runs were corrected based on the offset between measured and certified values for CRMs. Instrumental precision for the samples from this study was ±2.67 µmol/kg. pCO_2, calcite and aragonite saturations (Table 1) were determined from TA, temperature, pH_T and salinity data using the seacarb package

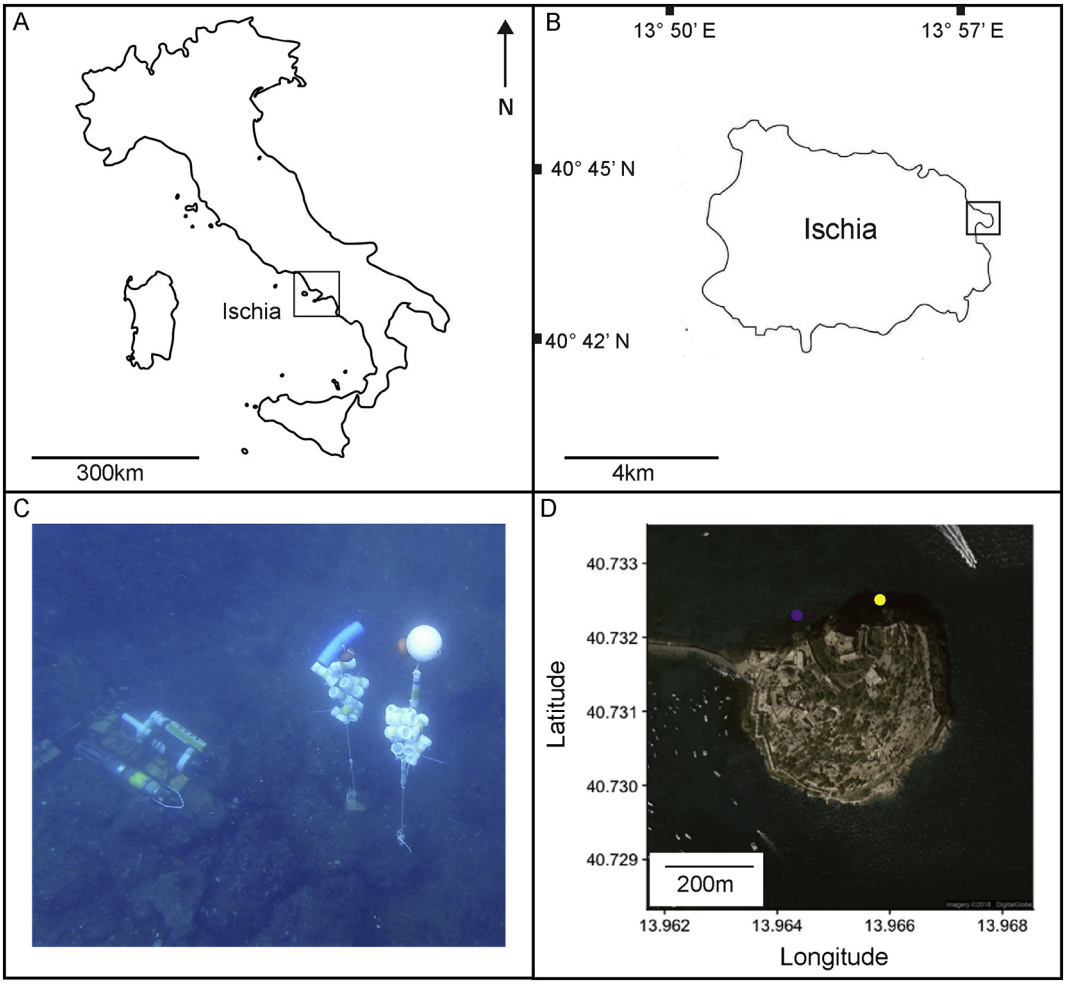

Fig. 3. Map of the *in situ* sites at the Castello Aragonese, located on the north-eastern coast of Ischia, Italy. Panel A shows the location of Ischia, with Panel B showing the location of the Castello Aragonese in Ischia. Panel C shows the configuration of sensor and larval home placement *in situ* (~3 m deep), cable tied to ropes and floating buoys which keep the homes in place. Panel D shows the Google earth aerial image of Castello Aragonese with the locations of the low pH (purple) and control (yellow) sites where larval homes and autonomous sensors were placed.

Table 1
Carbonate chemistry and water parameters in the laboratory and the field (mean ± standard error) during the experimental deployment when the embryos of *A. lixula* developed to the 2-armed echinopluteus. Minimum and maximum values are shown in brackets where relevant. For field data with autonomous logging, only data for the 24-hour experimental period are shown (see Table S1 for entire sensor deployment data) with significant differences between control and vent sites indicated by an *, analysed by a Wilcoxon or *t*-test.

	Laboratory		Field	
	Control pH	Low pH	Control	Vent
pH_T	8.01 ± 0.002[1] (8.01, 8.03)	7.80 ± 0.002[1] (7.79, 7.83)	7.95 ± 0.003* (7.87, 7.99)	7.80 ± 0.011* (7.33, 7.99)
pCO_2 (μatm)	472.59 (446. 25, 472.59)	837.52 (773.35, 859.95	564.66 ± 5.35 (499.17, 698.17)	901.09 ± 32.83 (509.17, 2744.93)
Ω calcite	5.18 (5.18, 5.37)	3.54 (3.47, 3.75)	4.57 ± 0.03 (3.93, 4.95)	3.58 ± 0.07 (1.31, 4.98)
Ω aragonite	3.42 (3.42, 3.55)	2.34 (2.30, 2.48)	3.02 ± 0.02 (3.26, 2.58)	2.36 ± 0.05 (0.86, 3.28)
Avg temperature (°C)	24.31 ± 0.03	24.78 ± 0.04	24.10 ± 0.01	24.07 ± 0.01
Tank 1	24.26 ± 0.00	24.86 ± 0.04	–	–
Tank 2	24.29 ± 0.00	24.50 ± 0.04	–	–
Tank 3	24.37 ± 0.08	24.97 ± 0.03	–	–
Temp above larval home	–	–	24.06 ± 0.00	24.03 ± 0.00
Temp below larval home	–	–	24.07 ± 0.00	24.04 ± 0.00
SeapHOx temp	–	–	24.16 ± 0.02	24.14 ± 0.02
Oxygen (%)	98.65 ± 0.38[1]	101.19 ± 0.74[1]	93.05 ± 0.93	98.61 ± 0.55
Oxygen (μmol/kg)	217.55 ± 4.48[1]	214.85 ± 1.48[1]	243.87 ± 2.34	258.82 ± 1.38
Salinity (psu)	38.40 ± 0.11[1]	38.33 ± 0.09[1]	38.22 ± 0.01*	37.80 ± 0.01*
Chl *a* (μg/L) YSI	–	–	0.49 ± 0.03*	0.66 ± 0.03*
Chl *a* (μg/L)[1]	0.15 ± 0.0004	0.14 ± 0.0004	0.22 ± 0.0009	0.29 ± 0.001
Total alkalinity (μmol/kg)[1]	2489.07 ± 1.21	2490.78 ± 2.17	2485.60 ± 13.35	2517.87 ± 20.01

[1] Note that for these discrete water samples, N = 4 in the field, N = 6 in the laboratory.

in R (Gattuso et al., 2015), using the dissociation constants of Millero et al., 2006.

2.6. Data analyses

2.6.1. Field site comparisons

As autonomous sensors recorded at different time intervals between the vent and control field sites, data for pH, salinity, oxygen, temperature and chl *a* were matched to the same timepoints so that equal sized datasets were compared between sites. Due to chl *a* sensor failure preceding larval home deployment, a section of the chl *a* sensor data for the control site was unusable. Thus, we used only data for the overlapping period that both sensors were functioning and matched data to the same timepoints between the two field sites. As data for each of these parameters were not normally distributed, we compared control and vent field measurements with non-parametric Wilcoxon Rank sum tests to test whether the mean values of each parameter were significantly different between field sites.

We also used the measurements of chl *a* and POC from the four discrete samples to better characterize the difference in food availability between the low pH and control treatments in both the field and the laboratory. For each setting (field and laboratory), we generated all 256 ($n = 4^4$) possible resampling combinations. Then we resampled both the low pH treatment and the control treatment and calculated the mean difference in the variable of interest (chl *a* or POC). This procedure resulted in 256 bootstrap estimates of the mean difference in chl *a* and POC between the low pH and control treatments in both the field and laboratory. We report the "food effect", the difference in food proxy (chl *a* or POC) between the low pH and control pH treatments for both the lab and the field, as the mean of this distribution of differences, along with the associated 2.5 and 97.5 bootstrap percentiles. Because we were interested in how low pH conditions may affect food availability, positive values of the estimated food difference indicate greater chl *a* or POC concentrations at the low pH condition relative to the control pH condition. The bootstrapping procedure described here is a good option for estimating effect sizes from small sample sets where one cannot make assumptions about normally distributed errors.

2.6.2. Larval growth — arm length

The mean arm length of the 30 larvae measured per larval home was taken as the datum. This meant that for each of the three populations of larvae, there were six replicates across each of the four treatments. To compare the effects of acidification on larval growth in the laboratory and field, we analysed arm length data with a three-way ANOVA with experiment type (laboratory or field), pH (low or control) and population (three populations) as fixed factors. To determine whether low pH affected body shape and normal arm growth we examined arm asymmetry. Using arm length data, the difference in length between the right and left arms was calculated as a measure of arm asymmetry. Arm asymmetry data were also analysed using a three-way ANOVA. Arm length and arm asymmetry data were checked for normality by inspecting Q-Q plots and heterogeneity through Levene's test prior to analysis, to confirm the assumptions of ANOVA were met. All statistical analyses were performed using R version 3.5.0 (R Core Team, 2013).

3. Results

3.1. Characterization of experimental conditions

In the field, mean pH_T differed significantly between sites (mean ± SE, control pH_T 7.95 ± 0.003; vent site pH_T 7.80 ± 0.011; Table 1; Fig. 4). Datasets for each sensor deployment are summarized in Table S1, and the conditions that coincided with the 24-hour experimental deployment are shown in Table 1. At the vent site pH fluctuated from pH_T 7.33–7.99 (Table 1; Fig. 4). Temperature was similar between the two sites (mean ± SE, control 24.10 ± 0.01; vent site 24.07 ± 0.01, Table 1, Fig. 4). SeapHOx and thermistors above and below the larval homes showed isothermal conditions in the water column (Table 1). Oxygen concentration was also similar between sites (mean ± SE, control 243.8 ± 2.3 μmol/kg; vent 258.8 ± 1.4 μmol/kg), but salinity significantly differed (mean ± SE, control 38.22 ± 0.01; vent 37.80 ± 0.01, Table 1).

Water velocity and flow direction did not differ greatly between field sites (Table S1, Figs. S2, S3), however a plot of horizontal depth-averaged velocities showed a difference in the primary directionality of flow between the two sites (Fig. S3). A time series of the depth-averaged horizontal velocities shows that the magnitudes of velocity are highly correlated between the two sites (Fig. S4). There was some divergence between the two field sites with slightly westward flow at the vent site and slightly eastward flow at the control site.

pH_T in the laboratory experiments was mean ± SE, 8.01 ± 0.002 and 7.80 ± 0.002 for the control and low pH treatments respectively (Table 1). Oxygen, salinity and temperature levels were similar between treatments (see Table 1).

3.2. Differences in food availability between sites

Comparison of sensor data from vent and control field sites show that chl *a* levels were significantly higher at the vent site compared with the control site ($p < 0.001$, W = 2,237,700, Table 1, Table S1, Fig. 4).

The discrete water samples from the field sites indicated the presence of greater food availability at the vent site relative to the control site, but similar food availability between laboratory control and low pH treatments (Table 1; Fig. S5). The mean and 95% confidence interval (2.5%, 97.5%) difference in chl *a* between the vent site and the control site was 0.757 μg/L (0.0349, 1.69). Considering that the mean chl *a* recorded by the YSI at the control field site was 0.49 ± 0.03, this mean difference in chl *a* indicates there is more than twice the amount of food availability at the vent site in comparison to the control site. The difference in POC between the vent site and control site was 135 μg/L (9.89, 302). The number (out of 256 possible occurrences) of bootstrap estimates less than zero (greater chl *a* or POC at the control site than at the vent site) were 5 and 1, for chl *a* and POC respectively. In contrast to the field data, the laboratory water samples do not provide strong evidence for greater food availability in the low pH treatment (Fig. S5). The mean (2.5%, 97.5%) difference in chl *a* between the low pH lab treatment and the control pH lab treatment was −0.104 μg/L (−0.363, 0.0509) and the mean difference in POC was −32.7 μg/L (−159, 93.7). The number of bootstrap estimates less than zero were 175 and 178 for chl *a* and POC, respectively.

3.3. Calcification of echinoplutei in the field and the laboratory

For the larvae deployed in the field, arm length was significantly longer in the low pH site (mean ± SE, 155 ± 2.51 μm) compared with the control site (mean ± SE, 144 ± 1.65 μm). In the laboratory, arm length was significantly smaller in low pH (mean ± SE, 131 ± 1.83 μm) compared to control pH (mean ± SE, 141 ± 1.82 μm). There was a significant effect of experiment type (field *vs* laboratory) ($p < 0.001$) and population ($p < 0.001$) on larval length (Table S2). The effect of pH differed depending on the experiment type ($p < 0.001$); in the field, larvae grew longer arms at the low pH site than at the control site. The opposite trend was seen in the laboratory, where larvae had shorter arms under low pH conditions (Table S2, Fig. 5). Experimental location (field *vs* laboratory) and pH each significantly interacted with population ($p = 0.0300$, $p = 0.0229$). Although all populations showed similar trends, *i.e.* in the field, larvae grew longer arms in low pH, and in the laboratory, larvae grew shorter arms in low pH, the magnitude of the pH effect differed.

Arm asymmetry was not affected by pH, but was by experiment type ($p = 0.04701$) and experiment type crossed with population ($p =$

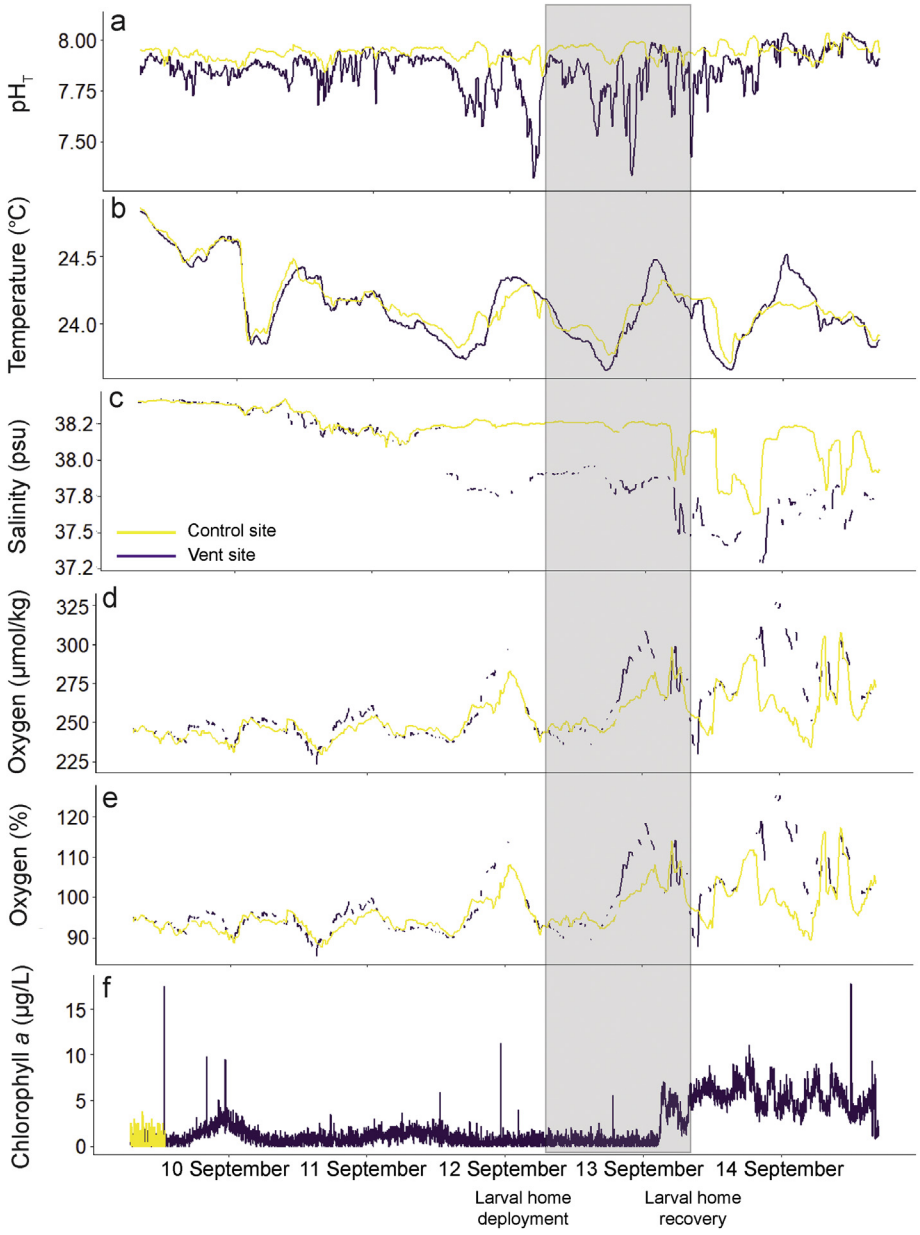

Fig. 4. a) pH_T b) temperature c) salinity d) dissolved oxygen concentration e) dissolved oxygen percent of saturation f) chl *a* sensor data. The grey box indicates the deployment period of the larvae in the field sites. Means and standard errors for the entire sensor deployment period are summarized in Table S1.

0.00425) (Table S2). However, this response was marginal as arm asymmetry was <4% across all treatments and populations (Table S2).

4. Discussion

Most OA studies on marine calcifiers have been conducted in a laboratory setting using stable pH conditions. The degree to which these results can be extrapolated to the natural environment is uncertain because replication of natural environmental variability in laboratory conditions is logistically challenging (Harvey et al., 2013; Ban et al., 2014; Nagelkerken and Munday, 2016). The aim of this study was to determine whether the effects of OA differed between laboratory and field studies, and we investigated this by examining the response of larvae to decreased pH in simultaneous laboratory and naturally acidified environments. We used the same populations of *A. lixula* embryos for laboratory and field deployments, which minimized confounding effects of parental source on arm length variability. Surprisingly, acidification had opposite effects on larval growth in the laboratory and in the field.

In agreement with the general stunting effect of low pH on the larval arms of echinoplutei (review by Byrne et al., 2013; Przeslawski et al., 2015; Byrne and Hernández, 2020), and previous laboratory studies with *A. lixula* (pH 7.7, Wangensteen et al., 2013; pH 7.7, Visconti et al., 2017; pH 7.4, García et al., 2018; pH 7.8, S. A. Foo unpubl.) we found reduced growth and calcification across all populations of larvae reared in stable low pH (pH_T 7.7) conditions in the laboratory. The presence of shorter, less calcified larvae under low pH in the laboratory is likely due to physiological stress on skeletogenesis and energetic constraints (Pörtner, 2010; Byrne et al., 2013; Stumpp et al., 2013).

In contrast to our laboratory findings, we found that larvae reared in fluctuating, low pH conditions (mean pH_T 7.7) at the vent site had the opposite response. All populations of larvae grew longer arms compared to their siblings reared at the control field site, as well as to those reared in the laboratory. The increased calcification seen in larvae reared at vent sites was not expected as they experienced low pH (7.33) during their field deployment, where at times, aragonite was undersaturated, a level where calcification in echinoplutei is unfavourable

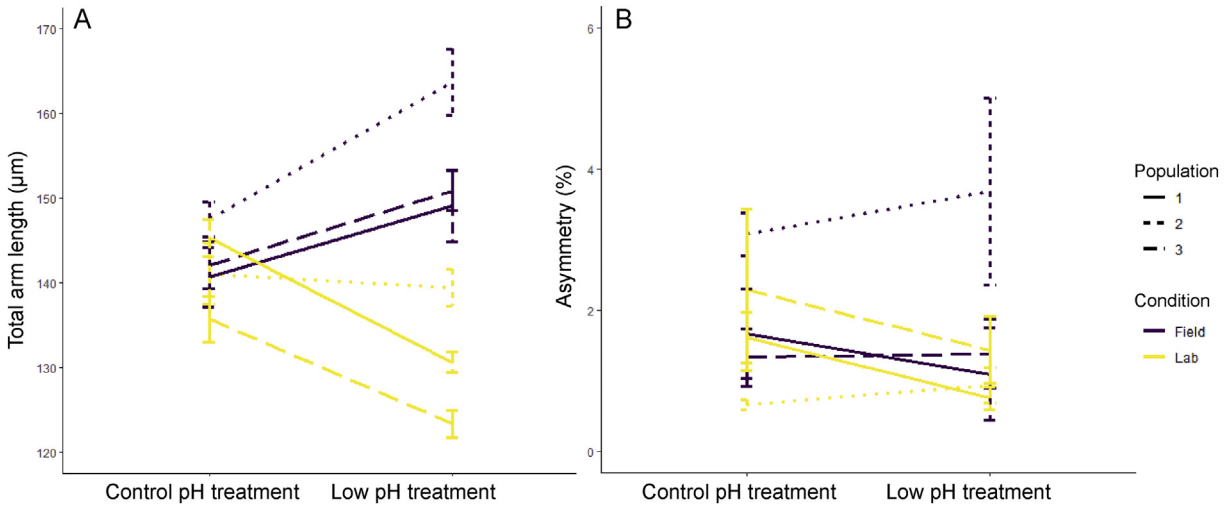

Fig. 5. Contrasting effects of low pH on sea urchin larvae reared in the laboratory and the field at 24 h for three larval populations. The plot shows the effects of pH treatments from field and laboratory conditions on A) mean total arm length, μm ± SE and B) arm length asymmetry.

(Byrne et al., 2013; Challener et al., 2013; Smith et al., 2016a, b). Additionally, studies on echinoplutei show that below pH 7.6, there is a sharp decrease in larval arm length (Byrne et al., 2013).

Measurement of a suite of oceanographic parameters at both field sites and monitoring of carbonate chemistry and nutrient levels across treatments allowed us to investigate some of the factors that may have contributed to the enhanced growth and calcification of the larvae at the vent site. During the time of larval home deployment, temperature and dissolved oxygen were similar in the field and the laboratory. Although salinity significantly differed between control and vent field sites, the mean difference of 0.42 psu is unlikely to have had a deleterious effect as sea urchin development is robust to small decreases in salinity. For example, for sea urchin larvae, survival probability only decreased when salinity was lowered to 27 psu (Sameoto and Metaxas, 2008). Similarly, survival and development are unaffected between changes in salinity between 30 and 40 psu in the sea urchin *Lytechinus variegatus* (Dominguez et al., 2007) with similar results seen across other echinoderms (Roller and Stickle, 1984; Schiopu and George, 2004).

At the field sites, there was little evidence for depth-dependent variation in salinity or temperature as indicated by the sensors placed at different depths. Similarly, flow speed, which can influence larval shape (Chan, 2012), was similar between control and low pH conditions. Food availability and pH fluctuations, however, differed between the vent site and the control site (as well as the laboratory treatments) with the vent site featuring higher food availability and greater pH variance. CO_2 enrichment of primary production, and correspondingly concentrations of chl *a* and POC, at the vent site would be expected and have been reported for other CO_2 vents in New Zealand and Papua New Guinea (Uthicke et al., 2016; Doubleday et al., 2019). This higher productivity indicates that there is more phytoplanktonic food for the larvae at the vent site.

Sea urchin larvae use the ciliary bands along their arms to feed where total food capture surface area is directly related to arm length (Strathmann et al., 1992; Strathmann and Grünbaum, 2006). When food is abundant in control pH conditions, echinoplutei display a short-armed phenotype to optimize allocation of energetic reserves to development of the juvenile rudiment and promote early settlement (Pedrotti and Fenaux, 1992; Soars et al., 2009). In low food environments, larvae produce longer arms to increase the chances of capturing food particles (Strathmann et al., 1992; Strathmann and Grünbaum, 2006; Fig. 6). Given higher food and low pH conditions at the vent site, it would be predicted that enhanced food would stimulate a plastic reduction in arm growth, and acidification would decrease calcification with the integrated response of the larvae resulting in a short arm phenotype, but this was not the case. The presence of the longest arms in the plutei of *A. lixula* reared at the vent site is counterintuitive (Fig. 6), especially because we know this species displays phenotypically plastic arm growth (Pedrotti and Fenaux, 1992).

It is possible that under fluctuating low pH levels, arm growth phenotypic plasticity is affected by energetic constraints or sensory inhibition. Exposure to decreased pH may influence arm growth plasticity due to the imperative to address the energetic deficit caused by acidification and metabolic stress. For the larvae of *Strongylocentrotus droebachiensis*, acidification (pH ≤ 7.7) results in a drop in gastric pH, compromising their ability to digest food (Stumpp et al., 2013). This results in decreased digestive efficiency, stimulating compensatory feeding (Stumpp et al., 2013). The larvae reared at the vent site may be exhibiting a trade-off to grow long arms and enhanced food particle capture to counter the energetic stress of acidification. This is congruent with the hypothesis that acidification stress in sea urchins is largely an energetic-hypercapnia issue (Stumpp et al., 2012, 2013; Byrne et al., 2013). Thus, a larger size may be an indirect effect of low pH. It is also possible that low pH interferes with larval sensory biology by impairing the feedback mechanism(s) between larval perception of environmental nutrients and the control of arm growth (Miner, 2007; Fig. 6).

Fluctuation in pH levels at the vent site may also have interfered or modulated the sensory ability of the larvae to integrate their environment with respect to plastic growth responses. There is mixed evidence for marine animal response to fluctuating pH. Larvae of the sea urchin *P. lividus* showed increased developmental rates under fluctuating, low pH levels (García et al., 2018), whereas diurnal pH fluctuations increased the variance in shell growth but not the mean levels of shell growth in the barnacle *Balanus improvisus* (Eriander et al., 2016). Similarly in our study, variance in arm length growth increased in the vent treatment in comparison to the control treatment (Fig. 5). pH sensitivity of larvae can also be intensified during development (Kapsenberg et al., 2018) and thus the timing of changes in pH during the 24-hour period that the larvae were at the vent site could have contributed to the differences in arm length observed. However, we obtained the same result in our preliminary pilot study undertaken in 2016, where larvae were exposed to a different variability regime (S. A. Foo unpubl.). Whether a similar response is found when rearing larvae in other fluctuating systems, *e.g.* tide pools and areas with upwelling, will help to distinguish whether the effects are due to fluctuating pH and/or increased food levels.

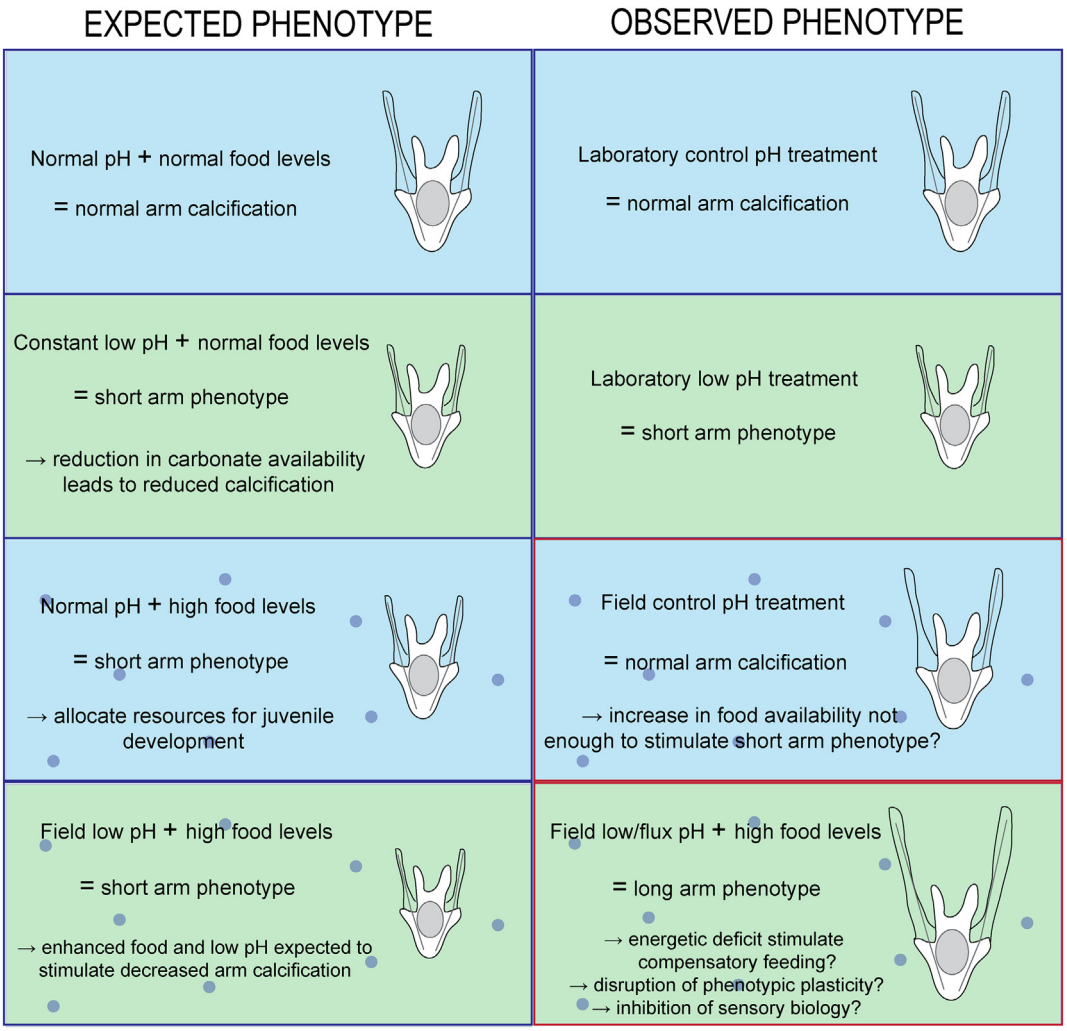

Fig. 6. Scenarios of pH and food levels and their effects on sea urchin larval development. Expected phenotypes from each scenario are displayed in comparison to the results observed in our treatments. When the outline colour matches, the observed phenotype agrees with the expected phenotype. Possible hypotheses are highlighted in the final panel to explain the phenotype displayed by the larvae in this experiment, counterintuitive to expectations.

In another study where sea urchin larvae were reared at a vent site (pH 7.50 to 7.72), the larvae of *Echinometra mathaii* had shorter arms than clutch mates deployed at a control site (pH 7.89 to 7.92; Lamare et al., 2016). This corresponds with expectations based on CO_2-dosing experiments with this species (Uthicke et al., 2016), but contrasts to our results with the larvae of *A. lixula*. In Uthicke et al., 2016, higher chl *a* levels at the vent site, *i.e.* increased phytoplankton levels, did not result in larvae with longer arms. It was concluded that pH effects were stronger than those of food availability (Lamare et al., 2016). As tropical larvae, the echinoplutei of *E. mathaii* may be better adapted to the oligotrophic coral reef environment, and so may have a different response to enhanced productivity than temperate sea urchin larvae. It is also not known if these larvae exhibit phenotypic plasticity in arm growth.

For *A. lixula* it appears that the planktonic phase of this species may be able to persist in a low pH environment through compensatory feeding, availing themseleves of the higher productivity stimulated by high CO_2. A similar response is seen in the adult stage of several herbivores resident at vent sites including sea urchins and gastropods (Uthicke et al., 2016; Connell et al., 2017; Doubleday et al., 2019). *Echinometra mathaii* resident at CO_2 vents (pH 7.89 to 7.92) are between two and three times larger than conspecifics living at normal control levels, a difference attributed to the higher algal food resources at the vent (Uthicke et al., 2016). As phenotypic plasticity underpins a species acclimatory response (Foo and Byrne, 2016), the plasticity displayed by *A. lixula* larvae may be crucial in buffering the population as a whole against the harmful effects of global change, buying time for genetic adaptation to occur. In conjunction with *A. lixula*'s thermophilic nature in both the larval and adult stage (Gianguzza, 2020), this species may be a winner in an acidifying ocean.

Our investigation of the effects of experimental and natural acidification on sibling larval populations, together with characterization of experimental conditions, increases our understanding of the response of sea urchin development under low pH. In light of the similarity of most environmental parameters, the results indicate that increased food availability, together with the modulating effect of fluctuating pH and modifications of sensory feedback mechanisms, may have contributed to increased arm growth in the *A. lixula* larvae. Our results demonstrate that interactions among acidification, food availability and environmental variability are likely to be complex with counterintuitive biological responses. After over a decade of OA studies on sea urchin larvae, with a variety of suggested response mechanisms (*e.g.* Byrne et al., 2013; Stumpp et al., 2013; Byrne and Hernández, 2020), our results highlight the need for controlled experiments with respect to food availability and application of naturally variable pH. Caution must be applied when drawing inferences from laboratory studies as our results show contrasting responses between laboratory and field settings for the effects of ocean acidification on sea urchin calcification within the same population of larvae.

CRediT authorship contribution statement

Shawna A. Foo: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing. **David A. Koweek:** Formal analysis, Investigation, Data curation, Writing - review & editing. **Marco Munari:** Investigation, Data curation, Writing - review & editing. **Maria Cristina Gambi:** Conceptualization, Methodology, Investigation, Data curation, Writing - review & editing. **Maria Byrne:** Conceptualization, Methodology, Investigation, Data curation, Writing - review & editing. **Ken Caldeira:** Investigation, Data curation, Writing - review & editing, Funding acquisition.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.138003.

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