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RESEARCH ARTICLE

Sea urchins in a high-CO_2 world: partitioned effects of body size, ocean warming and acidification on metabolic rate

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ABSTRACT

Body size and temperature are the major factors explaining metabolic rate, and the additional factor of pH is a major driver at the biochemical level. These three factors have frequently been found to interact, complicating the formulation of broad models predicting metabolic rates and hence ecological functioning. In this first study of the effects of warming and ocean acidification, and their potential interaction, on metabolic rate across a broad range in body size (two to three orders of magnitude difference in body mass), we addressed the impact of climate change on the sea urchin *Heliocidaris erythrogramma* in context with climate projections for southeast Australia, an ocean warming hotspot. Urchins were gradually introduced to two temperatures (18 and 23°C) and two pH levels (7.5 and 8.0), at which they were maintained for 2 months. Identical experimental trials separated by several weeks validated the fact that a new physiological steady state had been reached, otherwise known as acclimation. The relationship between body size, temperature and acidification on the metabolic rate of *H. erythrogramma* was strikingly stable. Both stressors caused increases in metabolic rate: 20% for temperature and 19% for pH. Combined effects were additive: a 44% increase in metabolism. Body size had a highly stable relationship with metabolic rate regardless of temperature or pH. None of these diverse drivers of metabolism interacted or modulated the effects of the others, highlighting the partitioned nature of how each influences metabolic rate, and the importance of achieving a full acclimation state. Despite these increases in energetic demand there was very limited capacity for compensatory modulating of feeding rate; food consumption increased only in the very smallest specimens, and only in response to temperature, and not pH. Our data show that warming, acidification and body size all substantially affect metabolism and are highly consistent and partitioned in their effects, and for *H. erythrogramma*, near-future climate change will incur a substantial energetic cost.

KEY WORDS: Metabolic scaling, Metabolism, *Heliocidaris erythrogramma*, Ocean acidification, Ocean warming, Acclimation

INTRODUCTION

The influence of biotic and abiotic factors on metabolism has long been of interest, both to elucidate the fundamental mechanisms determining metabolic rate and because metabolic changes are frequently accompanied by alterations to behaviour, survival, energetic demand and other factors affecting species' fitness and ecosystem functioning. Most of the variation in animal metabolic rate is explained by temperature and body size (Brown et al., 2004). Environmental temperature is a key driver of metabolism in ectotherms, because it controls body temperature, and thus cellular diffusion rates and enzyme kinematics (within physiological limits) (Newell and Northcroft, 1967).

The relationship between body size and metabolic rate has been intensely studied for decades (Agutter and Wheatley, 2004). Typically, metabolic rate (R) increases with increasing body size according to the power law $R=aM^b$, where M is body mass, a is the metabolic coefficient and b is a scaling exponent (Agutter and Wheatley, 2004). Historically, b was considered a fundamental physical property of equal value in all organisms, with proposed values including ⅔ (Rubner, 1883) and ¾ (Kleiber, 1947). More recently, substantial variation in b has been recognised, both between (Glazier, 2010) and within (Hirst et al., 2014) species. One factor causing intraspecific variation in the value of b is temperature (Carey and Sigwart, 2014; Killen et al., 2010), suggesting that body size and temperature can modulate the effect of each other.

pH also influences metabolism because of its direct effect upon biochemical pathways, and is particularly important in aquatic organisms, because the pH of the external medium can directly affect that of internal body tissues (Collard et al., 2015). Some organisms have good control over tissue acid–base balance (Collard et al., 2013b), while others have poor to no control (Catarino et al., 2012) or only under substantial energetic cost (Maas et al., 2012). In organisms such as echinoderms, corals and molluscs, reduced pH substantially increases the energetic cost of producing or maintaining calcium carbonate skeletons (Kaniewska et al., 2012), and so lower seawater pH [ocean acidification (OA)] is often accompanied by elevated metabolic rates (Beniash et al., 2010). However, in other organisms, OA has the opposite effect and is accompanied by metabolic depression (Collard et al., 2013a; Melatunan et al., 2011), because of direct effects upon metabolic pathways such as disruption to mitochondrial functioning (Kaniewska et al., 2012), or because it may conserve resources during periodic hypercapnic conditions (Christensen et al., 2011).

The combined effects of temperature and OA are unpredictable and frequently interact (Przeslawski et al., 2015). The additional influence of a fundamental biological parameter, body size, on responses to these stressors is rarely considered. Body size is important in modulating species' responses to both warming (Carey and Sigwart, 2014; Killen et al., 2010) and acidification (Appelhans et al., 2014; Waldbusser et al., 2010), as well as other stressors such as hypoxia (Clark et al., 2013). In addition, body sizes of species are predicted to decrease due to environmental warming (Horne et al., 2015) and acidification (Sommer et al., 2015), so the true relationship between body size and climate change stressors is a notable knowledge gap.

Although there are numerous studies investigating the isolated and combined effects of OA and temperature on metabolism in marine invertebrates, very few have incorporated body size as an

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List of symbols and abbreviations

a	mass coefficient
AFDM	ash-free dry mass
A_T	total alkalinity
b	metabolic scaling exponent
CRM	certified reference material
FSW	flow-through filtered seawater
L_m	predicted mass-specific metabolic rate at the $\log_{10}$ midpoint mass
M	body mass
OLS	ordinary least squares
pH_T	total pH
R	metabolic rate
r_F	feeding rate
TD	test diameter
V_{O_2}	routine oxygen consumption

additional explanatory variable. In addition, many studies only examine short-term responses due to brief maintenance durations (e.g. Carey et al., 2014), or because of immediate exposure to altered conditions (e.g. Stumpp et al., 2011; Suckling et al., 2015). The relevance of such studies in predicting long-term consequences is uncertain because they may represent 'shock' responses (Byrne, 2012; Queirós et al., 2015). Acclimation to altered conditions, that is, when physiological functioning has reached a new steady state (Suckling et al., 2015), may take an extensive period of time depending on the species. Insufficient exposure duration means that experiments may be conducted while the animal is still in a state of physiological transition (Munguia and Alenius, 2013; Suckling et al., 2015). Very few studies validate that physiological acclimation has been achieved by conducting identical experiments separated by an appropriate time period.

We investigated the effects of temperature, acidification and body size on metabolic rate in the sea urchin *Heliocidaris erythrogramma* (Valenciennes 1846), an ecologically important species in rocky reefs in eastern Australia (Keesing, 2013). Long-term maintenance began by gradually introducing specimens to experimental conditions, avoiding acute exposure. We validated that physiological equilibrium (i.e. acclimation) was achieved by conducting experimental trials separated by several weeks. Urchins are important components of marine ecosystems, with major ecological and carbon cycling roles, and are useful models to examine physiological responses to environmental change (Collard et al., 2015). In addition, many sea urchins, including *H. erythrogramma*, are abundant and grow relatively large in comparison to other benthic marine invertebrates, making them ideal for examining the effects of body size on metabolic responses.

In this study, we addressed the fundamental physiology of metabolic scaling in sea urchins, acclimating the widest size range so far examined in a single species. We addressed the potential consequences of climate change on *H. erythrogramma* in context with projections for southeast Australia, an ocean warming hotspot (Hobday and Lough, 2011). Warming is the most important present day stressor in the region because of both climate-driven increased poleward flow of a major western boundary current, and significant aerial warming of the Australian continent (IPCC, 2014). Urchins were gradually introduced to temperature and pH treatments representing near-future conditions over 4 weeks, followed by 2 months acclimation, to eliminate initial stress response and reduce as much as possible the influence of physiological history. Warming and acidification are known to cause metabolic changes in marine invertebrates, and in combination frequently act interactively (e.g. Catarino et al., 2012; Paganini et al., 2014). Body size has been shown to affect the magnitude of response to both of these stressors (Appelhans et al., 2014; Carey and Sigwart, 2014). Given these patterns, we expected that these three factors would interact to affect metabolic rate in *H. erythrogramma*.

MATERIALS AND METHODS

Collection and maintenance

Approximately 100 *H. erythrogramma* [11–80 mm test diameter (TD)] were collected from subtidal habitat in Little Bay, Sydney (33°58′S, 151°15′E), an open coast site, in June 2014 (ambient water temperatures ∼19–20°C). Urchins were immediately transported to the Sydney Institute of Marine Science and held in flow-through aquaria at ambient temperature (∼20°C). They were sorted into four groups of full size range of 25 specimens each. After 1 week, groups were transferred into a temperature-controlled room with a 12 h:12 h light:dark regime, and randomly allocated to one of four treatments in a two-way orthogonal design: two temperatures (18 and 23°C) and two pH levels [ambient total pH (pH_T) and −0.5 pH_T], distributed amongst two to three replicate tanks within each treatment such that total biomass was approximately equal in each tank (four to 15 specimens per tank). Tanks (32 litres) were supplied with flow-through filtered seawater (FSW, 20 µm, at 0.4 l min^{-1}). Airlines in each ensured mixing. Smaller specimens (<20 mm test diameter) were partitioned in plastic containers (2 litres) with mesh lids within tanks, each with an additional airline to ensure mixing. Tanks were monitored daily for dissolved oxygen (>95% at all times). Tanks were cleaned of faeces and fouling every 2 days.

After initial loss of some specimens within days of collection there was no mortality during acclimation; however, six specimens that showed indications of illness (dropped spines, reduced movement) were removed. These were not associated with a particular temperature or pH. Specimens were fed *ad libitum* on *Sargassum* spp. every 2 days. To ensure sufficient food availability, a large batch was collected, removed of macrofauna, dried at 50°C for 48 h and stored in double-sealed Ziploc bags containing a natural desiccant. Dried algae was submerged in seawater for ∼30 min to reconstitute before being used for feeding. Two weeks prior to the first respirometry experiments, sea urchins were transitioned to a diet of the same dried *Sargassum* powdered and suspended in solidified agar, and fed daily.

Temperature and seawater chemistry control and monitoring

Replicate tanks were maintained from 60 litre header tanks supplied with FSW. Temperature was controlled by a computer-controlled feedback system. To adjust pH, food-grade CO_2 (BOC Australia) was injected into CO_2-scrubbed ambient air using a VSO® thermally compensated low flow controller valve (Parker Hannifin, Mayfield Heights, OH, USA). A proportional–integral–derivative P_{CO_2} controller ensured precise CO_2 ppm, and the mixed air–CO_2 supply was bubbled continuously and vigorously into header tanks using 20 cm ceramic diffusers. Temperature was changed from ambient (∼20°C) by 1°C every 4 days until target temperatures (18 and 23°C) were reached. After four subsequent days, pH was decreased by 0.1 units every 4 days until target pH (7.6) was reached. All changes were gradual; header tanks took ∼1 h to reach new set-points, with several hours more for changes to propagate to treatment tanks. Achieving final conditions took 4 weeks, and sea urchins were maintained under these conditions for 2 months.

Treatment stability was monitored daily in each treatment tank using a WTW SenTix data logger and temperature/pH sensor

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(SenTix 940). The pH electrode was calibrated daily using NIST buffers pH 4, 7 and 10 (ProSciTech). Once final treatment conditions were achieved, pH_T was determined daily using the spectrophotometric m-cresol purple (mCP sodium salt pure, Acros Organics, Lot A0321770) method (Liu et al., 2011) using an Ocean Optics USB4000+ Miniature Fiber Optic Spectrometer with a tungsten light source. The pH was verified for consistency at the beginning of the experiment by measuring certified reference material (CRM) for CO_2 in seawater (Dickson et al., 2007) and comparing our pH values with those inferred from published total alkalinity (A_T) and total dissolved inorganic carbon values for the CRM. A_T was determined two to three times weekly by potentiometric titration (907 Titrando, Metrohm) using CRMs (Dickson et al., 2007). Precision and reproducibility of CRM A_T was assessed using 10 measurements made by the same user and equipment (s.d.=5.27). Carbonate system parameters were determined from pH_T and A_T using CO2Calc using the dissociation constants by Mehrbach et al. (1973) as refitted by Dickson and Millero (1987). Treatment system parameters were extremely stable and consistent after targets were achieved (Table 1).

Respirometry

Routine oxygen consumption (V_{O_2}; mg O_2 h^{-1}) was determined by intermittent-flow respirometry, and conducted twice for each specimen in weeks 8 and 11 after target conditions were reached. Specimens were fasted for 3 days before measurements and placed into circular respirometry chambers. Chambers were sealed and placed in a water bath positioned above magnetic stirring plates. A mesh platform separated the specimen from a rotating stir bar in the bottom of each chamber, ensuring mixing. An optical oxygen probe (Vernier, Beaverton, OR, USA) was inserted into each chamber. Chambers were supplied with FSW from the appropriate treatment header tank. This was allowed to flow through the respirometer to allow the specimen to become accustomed to the chamber, before the supply was halted and recording began. After an initial settling period, specimens were generally sedentary, and spontaneous movement was seldom observed. Oxygen concentration was recorded every second, and after a decrease of approximately 10% the chambers were flushed with fresh seawater and recordings were repeated. In the 8-week experiments, flushing occurred twice, giving three recordings for each specimen; the 11-week experiments were flushed once, giving two recordings for each specimen. Oxygen probes were calibrated daily and calibrations were checked after each individual trial. No substantial probe drift was observed. Control trials (n=20) using chambers containing only seawater were used to determine background microbial oxygen consumption, which was found to be negligible.

After each trial, chambers were dried externally and weighed. The specimen was removed and blotted dry, and wet mass was determined. These masses were subtracted from the total chamber mass to determine internal water mass, and this was converted to volume using the seawater density for the appropriate temperature and salinity. After the 8-week trial, specimens were returned to their original treatment tanks. After the 11-week trial, they were euthanised, dried at 60°C for 48 h, weighed, then incinerated in a muffle furnace at 500°C for 2 h and reweighed, the difference in these being ash-free dry mass (AFDM). Masses were determined using an analytical balance accurate to 0.0001 g.

Feeding trials

Feeding trials were conducted at 9 weeks, broadly following Beddingfield and McClintock (1998). Twenty-one specimens from each treatment were placed in individual open circular chambers supplied with FSW from the appropriate header tanks. Flow through each chamber differed based on specimen size; larger specimens were given greater flow to ensure their greater oxygen demand was met. Oxygen, temperature and pH were monitored daily within the chambers to ensure treatment consistency. Chamber size varied with specimen size (circumference×depth; 20×10, 20×9, 13×9, 11×7, 8×7 and 5.5×5 cm). Feeding trials lasted 6 days. The first 24 h period was used to allow the specimen to become accustomed to the chamber. Feed was prepared daily; dried algae was blended to a fine powder and mixed in the following proportions: 2 g algae, 5 g pure agar powder and 93 ml seawater. This mixture was set in a refrigerator and cut into different sized cubes for feeding. Urchins with a TD of >50, 20–50, 15–20 and <15 mm were provided different sized food cubes: 7.5×7.5, 5×5, 2.5×2.5 and 2×2 mm, respectively. The food was placed directly on and around specimens to minimise seek time, and none had problems manipulating the food. A pilot test determined how much specimens ate *ad libitum* in 24 h, and in the trials sea urchins were given approximately double this amount every day. The amount given to each specimen was weighed. After 24 h, any remaining food was removed and a new weighed supply was added. The leftover food was blotted dry and weighed. Difference in food mass between pre- and post-feeding allowed determination of food mass ingested in 24 h. This was repeated for 5 days, following which specimens were returned to their original treatment tank. Controls without sea urchins (n=56) determined that food did not substantially change mass in 24 h (mean±s.d. decrease of 3.1±1.1%). This small decrease was not associated with cube size, temperature or pH (three-way ANOVA; size, P=0.97; temperature, P=0.30; pH, P=0.30).

Data analyses

Each individual routine oxygen consumption trace was smoothed using a 30 to 60 s rolling average, and assessed visually for consistency. A representative section comprising approximately 30 min was selected within each run, and O_2 uptake rate over this section was determined. V_{O_2} was calculated as the average of the repeated recordings for each specimen. Metabolic rates were not significantly different between 8- and 11-week experiments (two-tailed paired t-test; t_{93}=1.06, P=0.29), indicating that physiological

Table 1. Carbonate system parameters (means±s.d.) in experimental treatments

Treatment	Temperature (°C)	pH_T	Salinity	A_T	P_{CO_2} (µatm)	C_T (µmol kg^{-1})	Ω_{Ca}	Ω_{Ar}
Low temperature, ambient pH	18.0±0.2	7.99±0.01	35	2262±6	458±1	2051±3	3.66±0.04	2.37±0.03
Low temperature, low pH	18.1±0.2	7.51±0.05	35	2262±9	1571±7	2231±8	1.36±0.01	0.88±0.01
High temperature, ambient pH	23.0±0.3	8.03±0.03	35	2260±7	405±1	1985±7	4.68±0.02	3.07±0.01
High temperature, low pH	23.0±0.3	7.56±0.05	35	2259±9	1389±4	2189±8	1.83±0.00	1.20±0.00

Number of measurements per treatment: temperature=72; pH_T=42; A_T=16. Total dissolved inorganic carbon (C_T), P_{CO_2} and calcium carbonate saturation state for calcite and aragonite (Ω_{Ca}, Ω_{Ar}) were calculated from total pH (pH_T) and total alkalinity (A_T) using CO2Calc.

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equilibrium had been achieved at the 8-week stage. The 8-week data were used for all subsequent analyses.

V_{O_2} and AFDM were $\log_{10}$ transformed and linear ordinary least squares (OLS) regression analysis was performed, giving mass–metabolism relationships for each treatment group (Table 2, Fig. 1). To compare slope (b) and elevation (a) of the resulting linear models, analysis of covariance (ANCOVA) was used with V_{O_2} as a dependent variable, AFDM as a primary independent variable, and temperature and pH as covariates. To test for tank effects, ANCOVAs were initially run with treatment containing the nested factor of tank identity, but no interactions with tank identity were observed and it was removed from subsequent analyses. No interactions were observed between mass and treatment (i.e. equal slopes), and so model simplification was performed by repeating ANCOVAs as additive models to more robustly test for differences in elevation (Table 3) (Crawley, 2007). Similar ANCOVA analyses were conducted on mean daily feeding rates (r_F) with mass as a covariate (Table 4).

To examine changes to metabolic rates at a standard mass, L_m was calculated (Killen et al., 2010) as the mass-specific metabolic rate predicted by the linear model at the $\log_{10}$ midpoint mass (Fig. 2). Q_{10} values were determined at the same $\log_{10}$ midpoint mass using the equation:

$$Q_{10} = \left(\frac{L_{mB}}{L_{mA}}\right)^{\frac{10}{T_B - T_A}},$$

where L_{mA} is L_m at the higher temperature (T_A), and L_{mB} that at the lower temperature (T_B).

All data met the homogeneity of variance and normality assumptions of ANCOVA (Bartlett and D'Agostino tests). All statistical analyses were implemented in R (R Core Development Team, 2015). Tests of significance were performed with α=0.05, and measurements of variability are ±95% confidence intervals (CI) based on standard error (s.e.).

RESULTS

All three factors, temperature, pH and body size, had significant and highly consistent effects on metabolic rate (R). The combined effect of temperature and pH were additive, and maintenance of the metabolic scaling slope (b) in all four treatments indicates that the metabolic responses were identical across body size. However, despite an increased metabolic rate of up to 44% in the combined treatment, feeding rate increased only in smaller specimens, and only in response to temperature.

Effect of body size on metabolism

The scaling exponent b did not vary between treatments with either temperature (ANCOVA; $F_{1,86}$=0.79, P=0.38) or pH ($F_{1,86}$=0.17, P=0.68). Slopes were equal between regression models, and no interactions were associated with body mass (M), indicating that the effect of body size upon metabolism was highly consistent and not influenced by the physical conditions, either in isolation or combined (Table 3). Similarly, the metabolic responses to pH and temperature were not modulated by body size; relative changes in metabolism were equal regardless of size (Fig. 1B).

Effect of temperature and pH on metabolism

Both increased temperature and low pH had highly positive effects on R (temperature, $F_{1,90}$=25.24, P<0.001; pH, $F_{1,90}$=33.91, P<0.001). Our treatments of a 5°C temperature increase and a 0.5 decrease in pH_T coincidentally increased R by around the same amount: 20% (±7.3) and 19% (±6.4), respectively (Fig. 2). When combined, the effects were additive: an increase of 44% (±14.5). The equal regression slopes indicate that these increases, both in isolation and combined, were equal regardless of size (Fig. 1B). Q_{10} values were 1.41 in ambient pH conditions and 1.44 in low pH, showing the equal effects of temperature in either pH treatment.

Effect of body size, temperature and pH on feeding rate

Feeding rate (r_F) was influenced by body size, scaling allometrically at values from 0.50 to 0.59 (Fig. 3, Table 4). Despite metabolic increases in both high temperatures and low pH, feeding rate was not affected by either parameter ($F_{1,75}$=2.089, P=0.153; pH, $F_{1,75}$=0.110, P=0.741), even in the combined treatment (Fig. 3). There was, however, an interaction between mass and temperature ($F_{1,75}$=4.283, P=0.042), indicating that smaller individuals increased their feeding rates at the high temperature level in both pH treatments (Fig. 3), resulting in lower scaling of consumption at 23°C (mean exponent 0.51) versus 18°C (mean exponent 0.58). Small individuals of 0.01 and 1.0 g increased feeding rates by ∼25% and 9%, respectively, at 23°C, with no increase in feeding in larger sea urchins (Fig. 3). It appears that the smaller individuals were able to increase feeding rates to compensate for higher R at higher temperatures, but not for higher R caused by low pH. Larger individuals did not compensate for increased R through increased consumption.

DISCUSSION

As the first assessment of the effects of temperature and pH on metabolism across a wide range in body size of a sea urchin, our results provide new insights into echinoderm physiology. The relationship between body size, temperature and acidification on metabolic rate in *H. erythrogramma* was strikingly stable. In contrast to expectations, increased temperature and acidification did not have an interactive effect, and body size did not exhibit any modulating effects on either stressor. This highlights the highly partitioned nature of these factors, and suggests that the mechanisms by which each influences metabolism are relatively separated within the physiological pathway. By contrast, other studies of marine invertebrates, albeit often with brief acclimation and narrow size ranges, suggest that the combined metabolic effects of temperature and pH are highly interactive (Carey and Sigwart, 2014; Matoo et al., 2013; Melatunan et al., 2011; Paganini et al., 2014; Uthicke et al., 2014). The increased energetic costs that are associated with a

Table 2. Experiment summary data, including sample size (N), mass range (ash-free dry mass) and linear model parameters (±s.e.) of the form $\log_{10}R=\log_{10}a+\log_{10}M \times b$

Treatment	N	Mass (g)	$\log_{10}a$	b	R^2	b=0.67	b=0.75	b=1.00
Low temperature, ambient pH	22	0.07–8.40	−0.053±0.012	0.72±0.02	0.99	**0.002**	0.105	**<0.000**
Low temperature, low pH	23	0.04–9.87	0.028±0.012	0.72±0.02	0.99	**0.002**	0.108	**<0.000**
High temperature, ambient pH	25	0.04–7.06	0.018±0.011	0.71±0.02	0.99	**0.005**	**0.023**	**<0.000**
High temperature, low pH	24	0.05–7.01	0.101±0.020	0.70±0.03	0.97	0.278	0.076	**<0.000**

R, metabolic rate (oxygen uptake, mg O_2 h^{-1}); M, ash-free dry mass (see also Fig. 1). The final three columns show results of tests for significant differences in slope b against commonly proposed values (significant differences indicated in bold; tested using the SMATR package for R).

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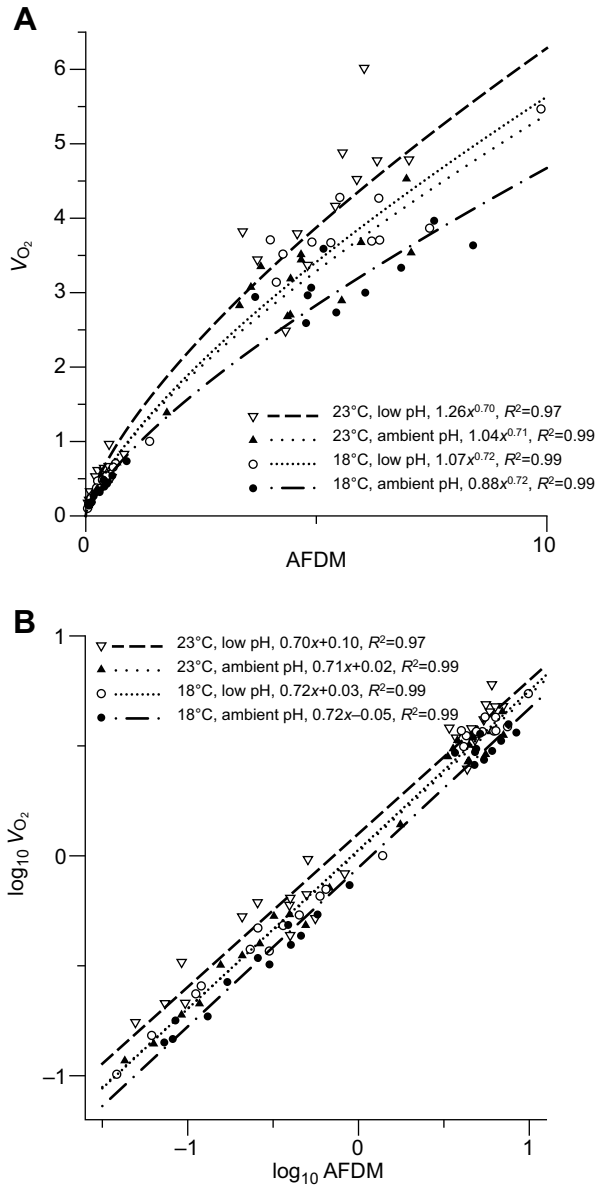

Fig. 1. Mass–metabolism relationships in the sea urchin *Heliocidaris erythrogramma* for the four experimental treatment groups. (A) Power regressions of the form $R=aM^b$, where R is metabolic rate, represented here as oxygen uptake rate (V_{O_2}; mg O_2 h^{-1}), M is mass, represented here as ash-free dry mass (AFDM; g), a is the mass coefficient or y-intercept, and b is the scaling exponent. Regressions equations are shown in the legend. (B) The same data represented as linear regressions on $\log_{10}$-transformed data. Regression equations here are of the form $\log_{10}R=b\times\log_{10}M+\log_{10}a$. Here, b represents the slope of the linear regression. Regression lines for the 23°C/ambient pH and 18°C/low pH treatments closely overlap and are partly obscured.

warmer, more acidic ocean could drastically affect the survival and distribution of marine invertebrates (Gaylord et al., 2015). Increases of 44% in metabolism in the combined treatments suggest that near-future climate change will result in a substantial increase in energetic costs in *H. erythrogramma*, and that this will affect all age classes within the species. These increased energetic costs could be minimised through acclimation or adaptation of the species over the coming decades, but it is likely to be subject to some permanent increased energetic demand, and as a primary grazer this could cause substantial ongoing ecological effects (Falkenberg et al., 2013).

Table 3. Analysis of covariance for metabolic rate (*R*) in *Heliocidaris erythrogramma* with body mass (ash-free dry mass) as primary independent variable (continuous), and temperature and pH as covariates (categorical, two levels of each)

Source of variation	d.f.	MS	*F*	*P*
Body mass	1	25.602	5335.111	**$<2\times10^{-16}$**
Temperature	1	0.117	24.433	**3.76×10^{-06}**
pH	1	0.158	32.829	**1.45×10^{-07}**
Body mass×temperature	1	0.004	0.793	0.376
Body mass×pH	1	0.001	0.167	0.684
Temperature×pH	1	0.000	0.002	0.964
Body mass×temperature×pH	1	0.001	0.166	0.684
Residuals	86	0.005		
Body mass	1	25.602	5510.95	**$<2\times10^{-16}$**
Temperature	1	0.117	25.24	**0.0000026**
pH	1	0.158	33.91	**0.0000001**
Residuals	90	0.005		

When no significant interactions were observed (i.e. regression models had equal slopes), to more robustly test the effect of each factor upon *R*, model simplification was performed by repeating the ANCOVA as an additive model (bottom four rows) (Crawley, 2007). Significant factors (P<0.05) affecting *R* are indicated in bold.

We saw very limited capacity for increased grazing under the increased energetic demand in response to warming and acidification. Higher temperatures typically cause higher feeding rates, but the opposite has been observed under low pH conditions in echinoderms, leading to reduced energy available for growth processes (e.g. Appelhans et al., 2014; Stumpp et al., 2012). The reasons for this apparent inability to increase feeding rate are uncertain; it may be some manner of functional constraint due to the architecture of the feeding parts only being able to process a certain volume of food. An alternative scenario is that consumption rate and actual energetic demand are uncoupled; that in the presence of ample food supply, *H. erythrogramma* acts as a 'conveyer-belt' feeder, ingesting more food than is actually required, and assimilation varies with metabolic requirements. Assimilation may also vary with other factors. Firstly, digestive transit time in sea urchins is highly variable, which could allow for differences in absorption efficiency; secondly, the full role of gut bacteria in absorption is not fully understood (Lawrence et al., 2013). However, our data suggest the possibility of a shortfall between increased metabolic requirements but limited ability to increase food acquisition. This was particularly notable in larger specimens, which would be expected to contribute most to trophic control. The consequences under near-future climate change for the rocky reef habitat in which *H. erythrogramma* are the dominant grazers remain uncertain. Our data suggest that smaller individuals may become more competitive for shared food resources at higher temperatures, and while the algal biomass consumed by smaller individuals is much less than that of larger individuals, there remain competitive implications. Algal communities are likely to be greatly altered under climate change, both through changes to trophic control through species impacts, and through direct effects of OA and warming (Harley et al., 2012). Changes to the ecological balance involving dominant grazers have the potential to alter community structure.

Body size

Body size is the most fundamental of organismal traits, affecting all aspects of biology and ecology. There are numerous, highly contested theoretical models that explain the mechanistic basis between body size and metabolism, and variability in the scaling

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Table 4. Analysis of covariance for feeding rate (r_F) in *H. erythrogramma* with body mass (AFDM) as primary independent variable (continuous), and temperature and pH as covariates (categorical, two levels of each)

Source of variation	d.f.	MS	*F*	*P*
Body mass	1	13.852	942.975	**$<2\times10^{-16}$**
Temperature	1	0.031	2.089	0.153
pH	1	0.002	0.110	0.741
Body mass×temperature	1	0.063	4.283	**0.042**
Body mass×pH	1	0.000	0.000	0.995
Temperature×pH	1	0.000	0.014	0.905
Body mass×temperature×pH	1	0.003	0.185	0.669
Residuals	75	0.015		

Significant factors ($P<0.05$) affecting r_F are indicated in bold.

exponent *b* (White and Kearney, 2014). These models largely fall into two major approaches: that allometric metabolic scaling is determined (1) by the geometries of internal resource transport networks (e.g. vascular systems) (Banavar et al., 2010; West et al., 1999), or (2) by the constraints that the surface area of exchange surfaces place upon the transfer of metabolic fuel and heat (Kooijman, 2010; Rubner, 1883), with recent work on aquatic invertebrates favouring this latter model (Glazier et al., 2015; Hirst et al., 2014). The scaling exponents we observed here (b=0.70 to 0.72; Table 2), do not provide strong support for any particular model. In fact, our data (Table 2) are more supportive of the older paradigm that *b* is a fundamental, invariant value somewhere close to ⅔ or ¾. Some of the surface-area-constraint models predict that the scaling exponent will decrease with increasing temperature, a result observed in other studies (Carey and Sigwart, 2014; Doyle et al., 2012; Killen et al., 2010; Weldon et al., 2013). Here, we observed slightly lower *b* values in higher temperatures, but this was not significant. Three other echinoderm species have been shown previously to have highly consistent *b* values and responses to both temperature and pH across body size (Carey et al., 2014). This apparent stability in metabolic scaling may be common to

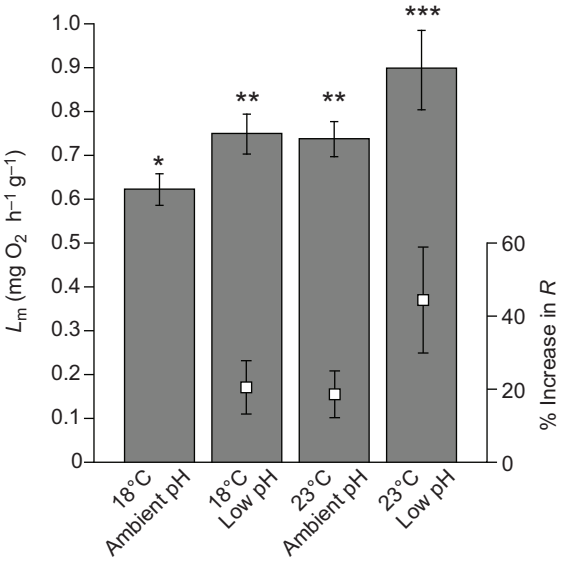

Fig. 2. The mass-specific metabolic rate predicted by the linear models at the midpoint mass in log space [L_m (±s.e.), left axis and bars]. Because of equal values of *b*, this plot would look similar at any particular chosen mass. Right axis and points show the same data expressed as percentage increase (±s.e.) in metabolic rate (*R*) above that in control (18°C/ambient pH) conditions at the same log midpoint mass.

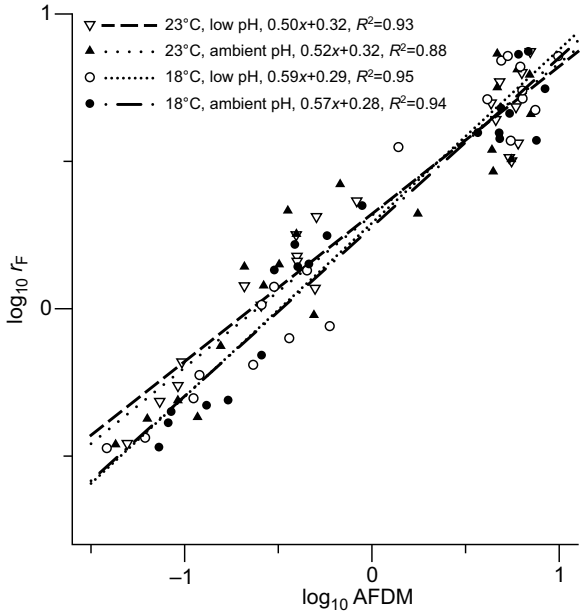

Fig. 3. Mass–feeding rate relationships for the four experimental treatment groups. Linear regressions on $\log_{10}$-transformed data of the form $\log_{10}r_F=b\times\log_{10}M+\log_{10}a$, where r_F is feeding rate of prepared agar food blocks (g day^{-1}), *M* is mass (ash-free dry mass, AFDM; g), *a* is the *y*-intercept and *b* is the scaling exponent of consumption. Regression lines for the two 23°C and two 18°C treatments closely overlap and are partly obscured.

echinoderms or related to some aspect of their biology, and suggests that, at least beyond vulnerable larval or juvenile stages, body size is not a factor in echinoderm responses to warming and acidification.

Responses to temperature and pH

While body size and temperature are the major determinants of metabolism (Brown et al., 2004), pH also influences metabolic rate through its effects upon cellular acid–base balance and ion transport (Kaniewska et al., 2012; Seibel et al., 2012). Low pH has also been shown to alter ATP allocation, even when overall metabolic rate remains unaltered (Pan et al., 2015). How lowered seawater pH causes changes to metabolic rates in marine invertebrates is complex and often species-specific, depending on which part of the metabolic pathway is affected (Carter et al., 2013; Pan et al., 2015). In sea urchins, the impacts of low pH may be minimal at the level of the organism, but cause a dramatic change to metabolic function at the cellular level (Pan et al., 2015) potentially compromising the energy available for biochemical functioning under environmental stress. A better understanding of the effects of acidification on the metabolic pathway is required to make better predictions as to how marine species will respond.

The effect of temperature was consistent across pH levels and with body size (Fig. 1). Q_{10} values (1.41 in ambient pH, 1.44 in low pH) were similar to those observed in other sea urchins (Watson et al., 2014) and echinoderms (Dame, 1972; Peck et al., 2008). Typical Q_{10} values for biochemical processes at optimum temperatures are between 2 and 3 (Lawrence, 1987), but lower values are common, and possibly associated with low sensitivity to temperature in calcifiers (Watson et al., 2014). The upper temperature used in the present study (23°C) was chosen to represent near-future mean temperatures likely to be experienced by this sea urchin in this region (Byrne et al., 2011; Hobday and Lough, 2011), but not so high as to cause obvious physiological

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impairment. From prior experiments, this occurs around 25°C, even in specimens collected around the annual summer maxima when temperature peaks of 25°C occur (M.B., personal observation). Adult *H. erythrogramma* do experience periodic exposure to temperatures well over this value in tidepools, as well as fluctuating pH (Wolfe et al., 2013). Therefore, the experimental conditions (23°C, pH 7.6) we used may already be experienced by *H. erythrogramma* on a periodic basis. However, the source population for this study was from the shallow subtidal, which is never emersed, and so their environmental history is unlikely to have involved marked daily temperature and/or pH fluctuations. Responses to brief, periodic exposures are also very different in character to ongoing responses to new mean conditions as examined in this study, and may represent an entirely different strategy of short-term resource conservation (Christensen et al., 2011; Parker et al., 2013). The dramatic increase in energetic demand of up to 44% after acclimation demonstrates that even modest changes in environmental conditions, such as those due in the next 100 years, could result in substantial ongoing energetic costs, although this may be moot if the more important factor is the ability to survive or successfully reproduce under greater periodic temperature extremes.

Temperature, body size and metabolism in sea urchins

The effects of temperature on R in sea urchins show the general trend in ectotherms of positive effects within physiological limits (Newell and Northcroft, 1967). These limits are often associated with natural temperature exposures (Lawrence, 1987 and references therein). The majority of studies do not, however, examine a substantial size range. One study examined energetics in two size classes of *Psammechinus miliaris* (8–16 and 29–37 mm) and noted differences in metabolic rate based on food source, but did not report on metabolic scaling (Otero-Villanueva et al., 2004). A study on seasonal variation in metabolism in the Antarctic sea urchin *Sterechinus neumayeri* examined a wide size range (3.8 to 59.9 mm diameter), but calculated R for a standard mass, and also did not report on scaling of metabolism or whether there was any difference in response to environmental temperature of different size classes (Brockington and Peck, 2001).

Only one prior study has examined metabolic rate in sea urchins across a relatively large size range and reported metabolic scaling exponents (Watson et al., 2014). This study found substantial differences in both R and b value in two sea urchins based on latitude, and hence environmental temperature (b=0.74 in the temperate species *P. miliaris*, and b=1.02 in the Antarctic species *S. neumayeri*), explained as a potentially plastic response of metabolic scaling to temperature, as predicted under the metabolic-level boundaries hypothesis (Glazier, 2010; Watson et al., 2014). Under this explanation, our b values (0.72–0.70) are consistent with those of Watson et al. (2014); as an echinoid living at an even warmer, lower latitude, *H. erythrogramma* would be expected to have a lower b value. A promising avenue for further research would be to determine b for tropical echinoids, which, based on this hypothesis (Glazier, 2010; Watson et al., 2014), should have values lower than this, approaching 0.67. However, estimations for b are extremely sensitive to the size range used, and here we used the widest size range that was practical (×237 ratio in mass). The two species examined in Watson et al. (2014) used an approximately ×17 and ×33 ratio between the smallest and largest specimens. Generally, mass ranges of over two orders of magnitude provide the most robust comparisons of allometric relationships (Sokal and Rohlf, 1995). No studies have examined metabolism in a comparable size range in sea urchins, and to our knowledge only one study on echinoderms has used a greater size range (×566), but with a limited acclimation duration of 1 week (Carey et al., 2014). Our dataset therefore provides the best estimation of scaling of metabolism in echinoderms to date.

Effects of ocean acidification on sea urchin metabolism

Only a few studies have examined the effects of OA on R in sea urchins. In larvae, two studies found OA caused increased R (Dorey et al., 2013; Stumpp et al., 2011). In one other, OA caused increased R in unfed larvae; in fed larvae there was no effect upon R but there were alterations to the allocation of metabolic energy (ATP) (Pan et al., 2015). Altered energy budgets unaccompanied by altered R have also been observed in adult sea urchins (Stumpp et al., 2012). Larval studies, however, cannot incorporate long acclimation periods, and so early-stage larvae or gametes are typically transferred immediately to low pH from ambient conditions (e.g. Dorey et al., 2013; Stumpp et al., 2012), so effects could be explained by short-term, 'shock' responses (Byrne, 2012; Munguia and Alenius, 2013).

The few studies on R in adult sea urchins exposed to OA found either increases or no effect (Kurihara et al., 2013; Moulin et al., 2015; Stumpp et al., 2012; Suckling et al., 2015). A study of the tropical sea urchin *Echinometra mathaei* found no effect on respiration after long-term acclimation to moderate low pH (Moulin et al., 2015), although R measurements were not ideal for comparison because specimens were not tested individually, not fasted and wet weight was used as the normalising mass metric. The Antarctic species *S. neumayeri* showed initial elevated R under low pH, but after long-term acclimation (2 years) no significant difference was observed (Suckling et al., 2015). Three studies found no effect of OA upon R in adult sea urchins over various acclimation durations (9 months, Kurihara et al., 2013; 13 months, Moulin et al., 2015; 10 and 45 days, Stumpp et al., 2011). However, two of these (Kurihara et al., 2013; Stumpp et al., 2012) reported substantial alterations to energy budgets, suggesting added energetic costs under OA, and so less available energy for processes such as growth and reproduction. Moderate stress caused by low pH may induce higher metabolic rates to supply increased energetic demands, but under more extreme conditions such increases in metabolism may be insufficient, and instead the animal will enter a state of metabolic depression to conserve resources (Christensen et al., 2011; Parker et al., 2013). Such a response is more suggestive of a shock response to rapid changes and is unlikely to be sustainable in a permanently changed environment. The more likely scenarios under permanent lower pH are substantial increases in energetic demand and metabolic rates, particularly in calcifying organisms (Kaniewska et al., 2012). Energetic limitation, such as under inadequate food supply, can have a substantial effect upon responses to OA; positive, neutral and negative metabolic responses can all be observed with similar magnitude changes in pH when under energy-limiting conditions (Gianguzza et al., 2014). This highlights the need for more holistic models of species energetics under combined stressors, incorporating factors such as energy available for growth, responses under energetic limitation, whether the baseline metabolism is close to optimal, and attainment of physiological equilibrium through adequate acclimation (Gianguzza et al., 2014; Pan et al., 2015). We saw very limited ability to increase resource consumption even with an increase of metabolic rate of 44%. This could lead to ongoing energetic trade-offs in *H. erythrogramma* between the needs of routine metabolism and the energy needed for important processes such as growth and reproduction.

The effect of combined warming and acidification on R in sea urchins has been investigated in two studies (Catarino et al., 2012;

Uthicke et al., 2014), but metabolic scaling across body size was not incorporated. Both of these studies found the effects of temperature and OA to be interactive. After 69 days exposure, the tropical sea urchin *Echinometra* sp. A (Uthicke et al., 2014) was found to exhibit no metabolic response to warming or OA in isolation, but there was a slight increase (5.9%) when these were combined. However, the treatments used were moderate (+2–3°C and −0.2 pH units). An Atlantic temperate species *Paracentrotus lividus* showed an increased *R* under OA, but only in cold temperatures, and not in the warm treatment (Catarino et al., 2012). This study used a relatively short maintenance period of 19 days. Our study is the first to show elevated *R* remaining after physiological acclimation, and that there was no interactive effect between temperature and pH. We also incorporated the additional variable of body size, showing that it too did not interact or modulate the effect of OA or warming upon *R*.

Physiological acclimation

Few studies of multiple stressor effects demonstrate that physiological acclimation, that is, reversion to a new physiological steady state, has been achieved by repeating experiments separated by an appropriate time period. The two exceptions to this for sea urchins (Moulin et al., 2015; Suckling et al., 2015) demonstrated that this can take weeks or even months. As well as duration, the rate of introduction to altered conditions can be a major factor (Byrne, 2012; Munguia and Alenius, 2013). Here, we took care to change treatment conditions both separately (temperature first, followed by pH) and gradually. Introduction to new conditions is often acute (e.g. Pan et al., 2015) or done over a brief period (e.g. 3 days, Suckling et al., 2015). Rapid introduction to altered pH or temperature may cause animals to be evaluated when in physiological transition, or not yet at a new steady state in their physiology (Cornwall and Hurd, 2015; Hochachka and Somero, 2002; Munguia and Alenius, 2013; Pörtner, 2008; Suckling et al., 2015). Our results highlight the importance of validating that acclimation has occurred over a suitable duration to achieve a new physiological steady state, and of introducing new conditions gradually to avoid stress responses.

Conclusions

Our study is the first to examine in combination the three major drivers of metabolic rate in marine ectotherms after sufficient acclimation, and provides data to support the unexpected result that temperature, low pH and body size did not exert an interactive effect on metabolism in *H. erythrogramma*. This highlights that these diverse drivers of metabolic rate can be highly partitioned in their effects. Despite substantial increased energetic demand, feeding rate increased only in smaller individuals, and only in response to temperature. The moderate changes to environmental conditions expected under near-future climate change will come with a substantial energetic cost to this sea urchin species, and an apparent inability to modulate feeding rate means that this cost may not be met.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

N.C., J.H. and M.B. conceived and designed the study. N.C. and J.H. conducted the experiments. N.C. analysed the data and wrote the first draft of the manuscript, and all authors contributed substantially to further versions.

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