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# One size fits all: stability of metabolic scaling under warming and ocean acidification in echinoderms

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**Abstract** Responses by marine species to ocean acidification (OA) have recently been shown to be modulated by external factors including temperature, food supply and salinity. However the role of a fundamental biological parameter relevant to all organisms, that of body size, in governing responses to multiple stressors has been almost entirely overlooked. Recent consensus suggests allometric scaling of metabolism with body size differs between species, the commonly cited 'universal' mass scaling exponent (*b*) of ¾ representing an average of exponents that naturally vary. One model, the Metabolic-Level Boundaries hypothesis, provides a testable prediction: that *b* will decrease within species under increasing temperature. However, no previous studies have examined how metabolic scaling may be directly affected by OA. We acclimated a wide body-mass range of three common NE Atlantic echinoderms (the sea star *Asterias rubens*, the brittlestars *Ophiothrix fragilis* and *Amphiura filiformis*) to two levels of $pCO_2$ and three temperatures, and metabolic rates were determined using closed-chamber respirometry. The results show that contrary to some models these echinoderm species possess a notable degree of stability in metabolic scaling under different abiotic conditions; the mass scaling exponent (*b*) varied in value between species, but not within species under different conditions. Additionally, we found no effect of OA on metabolic rates in any species. These data suggest responses to abiotic stressors are not modulated by body size in these species, as reflected in the stability of the metabolic scaling relationship. Such equivalence in response across ontogenetic size ranges has important implications for the stability of ecological food webs.

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## Introduction

In the marine environment, the most serious anthropogenic stressors are a consequence of increasing atmospheric $CO_2$, which has two major impacts; increased retention of heat in the atmosphere, the great majority of which is subsequently passed to the oceans (Bindoff et al. 2007); and absorption of increased amounts of $CO_2$ at the sea surface, forming carbonic acid and decreasing seawater pH, a process commonly known as ocean acidification (OA) (Doney et al. 2009). While major changes to temperature and ocean pH have occurred over geological timescales, the present changes are unprecedented in their rapidity (Doney et al. 2009; Diffenbaugh and Field 2013), and there remains substantial uncertainty as to how natural communities may be affected, and the potential for species to adapt to new conditions. Of these two facets, OA may be the more pervasive; many organisms, at least those in temperate and tropical regions, may avoid higher temperatures by shifting their ranges polewards towards colder latitudes (Wernberg et al. 2012), or through vertical migration to deeper, colder waters (Perry et al. 2005). Though seawater pH is highly variable on a regional scale (McElhany and Busch

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2013), OA will be a ubiquitous condition, and through vertical mixing of the oceans will eventually affect the entire marine ecosystem.

Physiological responses of species to these combined stressors will determine future survival, distribution, and ecological interactions (Pörtner and Farrell 2008; Widdicombe and Spicer 2008; Kelly and Hofmann 2012). The great majority of organisms in the marine environment are ectothermic, their metabolic rates chiefly determined by two factors; the temperature of the surrounding seawater, and their body size. Higher metabolic rates require organisms to increase their uptake of resources, and a shortfall between metabolic requirements and resource intake will lead to trade-offs between physiological processes requiring energy. Such trade-offs may lead to reduced fitness and survival (Morley et al. 2009). In addition, an increase in metabolism, and subsequent increased requirement for oxygen, may lead to reduction in aerobic scope (Morley et al. 2009), and may also be compounded by the fact that warmer water holds less dissolved oxygen (Clark et al. 2013).

The other major factor affecting metabolic rate is body size. How metabolism changes with body size has been a subject of much historical debate (Agutter and Wheatley 2004). Metabolic rates ($R$) typically increase relative to organismal body size according to the power function $R = aM^b$, where $M$ is body mass, $a$ is a normalisation coefficient which varies with metabolic level, and $b$ is a scaling exponent (Krogh 1916; Kleiber 1932; Bokma 2004). The same relationship can be represented as a linear regression with slope $b$ on a log–log plot of $M$ against $R$ (Glazier 2010). The value of $b$ has historically been considered a fundamental property related to internal body design (Kleiber 1947; Schmidt-Nielsen 1984; West et al. 1997), and much of the debate surrounding metabolic scaling has centred on different values for $b$, and the various models proposed to explain these values. Rubner (1883) suggested two-thirds (0.667), based on the surface-area-to-volume ratio of three-dimensional objects. However, the value of three-quarters (0.75) was more commonly observed in empirical studies (Kleiber 1932; Hemmingsen 1960; Peters 1983). West et al. (1997) proposed a prominent theoretical framework explaining the value of 0.75 based on the fractal nature of biological resource distribution networks (the WBE model), but this model has been the subject of much criticism for its broad assumptions and reductionism of biological complexity (Dodds et al. 2001; Darveau et al. 2002; Symonds and Elgar 2002; White and Seymour 2003; Agutter and Wheatley 2004; Bokma 2004; Suarez et al. 2004; Savage et al. 2008; White and Kearney 2013).

There appears however to be much variability around the value of 0.75 (Glazier 2005), and this value may simply represent an interspecies 'average' of a variable physiological parameter (Riisgård 1998; Agutter and Wheatley 2004; Bokma 2004; Suarez et al. 2004; Glazier 2006; Seibel 2007; Duncan et al. 2007; Brey 2010; White 2011). The value of $b$ may have a systematic variation within certain boundaries related to physiological, ecological and environmental factors (White and Kearney 2013). One proposed model, the Metabolic-Level Boundaries (MLB) hypothesis (Glazier 2005, 2010), suggests $b$ varies between 0.667 and 1.0 according to metabolic and activity levels of organisms. Other models, such as the 'cell size' (Kozłowski et al. 2003), and 'dynamic energy budget' (Kooijman 2010) models also predict a 0.667–1.0 variation in scaling exponents. Many of the competing models (though they are not necessarily exclusive of each other) predict similar boundary values and variability for $b$, and many also predict a central tendency close to 0.75, which means experimental tests of particular models are problematic (White and Kearney 2013). One prediction of the MLB model however, is that because of the exponential effect of temperature on metabolism in ectotherms, as temperature increases the value of $b$ should decrease (Killen et al. 2010; Ohlberger et al. 2012). Killen et al. (2010) showed support for this prediction interspecifically through meta-analysis on fish species. A negative temperature dependency of $b$ has also been observed in some experimental studies (Luo and Wang 2012; Ohlberger et al. 2012; Doyle et al. 2012; Weldon et al. 2013). This is not a universal pattern however, and the relationship between temperature and scaling has been found to be highly variable (Glazier 2005).

Models such as the MLB provide a direct mechanistic link between ecology and metabolic scaling, because an ecological factor affecting routine metabolism could potentially affect the value of $b$ (Killen et al. 2010). Metabolic rates (and hence body size) have been used to explain broad scale patterns in ecology, such as in the Metabolic Theory of Ecology (MTE) (Brown et al. 2004). Such large-scale predictive models frequently use single 'universal' scaling values (most commonly 0.75), effectively under the assumption that there is no natural variation in $b$. In addition, such models assume that the effects of body size and temperature on metabolism are independent of each other (Killen et al. 2010; Ohlberger et al. 2012). If, as the MLB suggests, there is an interaction between temperature and metabolic scaling, such models will require revision.

The effect of ocean acidification (OA) on metabolism, if one is observed, varies between species (Kroeker et al. 2013), and even within species under different combinations of other stressors (e.g. Christensen et al. 2011). Therefore, unlike with temperature, we lack a theoretical framework for predicting its direct effect on metabolic scaling. One previous study, on a freshwater fish, has examined changes to metabolic allometry under a severe pH change

**Table 1** Carbonate system speciation (±SD) in experimental treatments

| Treatment | $T$ (°C) | $pH_T$ | S | $A_T$ | $pCO_2$ (μ atm) | $C_T$ (μmol/kg SW) | $\Omega_{Ca}$ | $\Omega_{Ar}$ |
|---|---|---|---|---|---|---|---|---|
| Low $T$, control $pCO_2$ | 9.4 ± 0.1 | 8.03 ± 0.07 | 32 | 2,294 ± 27 | 428 ± 73 | 2,137 ± 41 | 2.90 ± 0.37 | 1.83 ± 0.23 |
| Low $T$, elevated $pCO_2$ | 9.4 ± 0.0 | 7.47 ± 0.09 | 32 | 2,293 ± 52 | 1,736 ± 368 | 2,317 ± 55 | 0.88 ± 0.17 | 0.56 ± 0.11 |
| Medium $T$, control $pCO_2$ | 15.6 ± 0.1 | 8.02 ± 0.10 | 32 | 2,289 ± 31 | 446 ± 102 | 2,093 ± 58 | 3.56 ± 0.69 | 2.27 ± 0.44 |
| Medium $T$, elevated $pCO_2$ | 15.5 ± 0.2 | 7.42 ± 0.11 | 32 | 2,291 ± 23 | 2,024 ± 487 | 2,306 ± 40 | 1.02 ± 0.25 | 0.65 ± 0.16 |
| High $T$, control $pCO_2$ | 20.3 ± 0.1 | 7.99 ± 0.09 | 32 | 2,289 ± 21 | 484 ± 119 | 2,074 ± 49 | 3.92 ± 0.70 | 2.54 ± 0.45 |
| High $T$, elevated $pCO_2$ | 20.3 ± 0.2 | 7.44 ± 0.12 | 32 | 2,291 ± 35 | 1,992 ± 503 | 2,281 ± 51 | 1.27 ± 0.34 | 0.82 ± 0.22 |

Total dissolved inorganic carbon ($C_T$), $pCO_2$ and calcium carbonate saturation state for calcite and aragonite ($\Omega_{Ca}$ $\Omega_{Ar}$) were calculated from $pH_T$ and total alkalinity ($A_T$) using CO2Calc

of 3.5 units (Vaca and White 2010), however the present study is the first to consider the direct effects on metabolic scaling of the complex changes to seawater carbonate chemistry under OA.

Echinoderms are major components of the marine ecosystem (Dupont et al. 2010), often shaping ecological assemblages (Paine 1966) and dominating benthic community biomass (Hughes et al. 2011). Warming and OA have shown generally negative impacts to development and survival in echinoderm larvae (reviewed in Byrne et al. 2013; Dupont and Thorndyke 2013), but effects on adults appear to be variable (Christensen et al. 2011). Here we examine the effects of temperature and $pCO_2$ on metabolism in three common Atlantic asteroid and ophiuroid species. Using broad body size ranges, we aim to determine metabolic scaling ($b$) in these species, whether scaling is variable under different conditions, and test the specific prediction of the MLB hypothesis that the value of $b$ decreases with increasing temperature.

## Materials and methods

Three common eastern Atlantic echinoderm species were chosen for experiments; the sea star *Asterias rubens* (Linnaeus, 1758), the brittlestars *Ophiothrix fragilis* (Abildgaard, 1789) and *Amphiura filiformis* (O F Müller, 1776). All specimens were collected by dredge in Gullmar Fjord, Sweden, and held in natural flowthrough seawater (salinity 32 ‰) at the Sven Lovén Centre for Marine Sciences, Kristineberg, University of Gothenburg. Individuals missing arms or undergoing arm regeneration were excluded. Within each species, specimens were sorted into six treatment groups of approximately 20–25 individuals representing wide body size ranges. Each group was allocated to one of six treatments (2 $pCO_2$ × 3 temperatures): control $pCO_2$ (~pH 8.0), or elevated $pCO_2$ (~pH 7.5); in low (~10 °C), medium (~15 °C), and high (~20 °C) temperatures. All experimental animals were acclimated to treatment conditions for 1 week while starved, prior to experimental recordings. Each treatment comprised two replicate 20 L tanks, and the treatment specimen group was divided randomly between the two tanks. Temperature was maintained through a temperature-controlled seawater supply (±0.2 °C). Seawater carbonate chemistry was maintained using a computerised feedback system (AquaMedic, Bissendorf, Germany) that regulates pH by addition of pure gaseous $CO_2$ directly into the seawater (±0.02 pH units). The apparatus was contained in a constant temperature facility that maintained air and seawater temperature, and temperature was recorded daily. Seawater samples for $pH_T$ (total scale) and total alkalinity ($A_T$) were collected twice weekly. $A_T$ was determined on filtered water samples with a titration system (Titroline Alpha Plus, SI Analytics). $pH_T$ was measured using a Metrohm (827 pH lab) electrode and were adjusted for pH measurements on the total scale using Tris (Tris/HCl) and AMP (2-aminopyridine/HCl) buffer solutions with a salinity of 32 (provided by Unité d'Océanographie Chimique, Université de Liège, Belgium). Seawater carbonate system speciation (Table 1) was calculated from $pH_T$ and $A_T$ with CO2Calc (version 1.0 for Mac OS X) using the dissociation constants by Mehrbach et al. (1973) as refitted by Dickson and Millero (1987).

After 1 week of acclimation, respiratory rates ($VO_2$, $mgO_2$ $h^{-1}$) and subsequently ash-free dry mass (AFDM, g) of each specimen were determined as per the protocol described in Carey et al. (2013). Briefly, specimens were sealed in perspex respirometry chambers fitted with small stirbars and placed in a waterbath at the appropriate treatment temperature situated above magnetic stirring plates. Oxygen concentrations were recorded at one-second intervals using optical oxygen probes (FOXY system, Ocean Optics, Dunedin, FL, USA). Recordings from chambers with seawater but no specimen were used to quantify background rates of oxygen consumption by microbial action, and a correction based on these was applied to experimental calculations. After experiments, specimens were dried at 60 °C for 24 h and weighed, then incinerated in a muffle furnace at 500 °C for two hours and reweighed, the difference in mass representing ash-free

**Table 2** Experiment summary data

| Species | Temperature treatment | $pCO_2$ treatment | *n* | Mass range | ln *a* | *b* | *R* | 0.667 | 0.75 | 1.00 |
|---|---|---|---|---|---|---|---|---|---|---|
| *Asterias rubens* | Low | Control | 20 | 0.043–5.660 | −1.29 ± 0.08 | 0.76 ± 0.05 | 0.99 | $P = 0.002$ | NS | $P < 0.001$ |
| | | Elevated | 23 | 0.019–5.575 | −1.22 ± 0.07 | 0.85 ± 0.04 | 0.99 | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |
| | Medium | Control | 25 | 0.028–5.421 | −0.83 ± 0.10 | 0.90 ± 0.05 | 0.99 | $P < 0.001$ | $P < 0.001$ | $P = 0.001$ |
| | | Elevated | 22 | 0.010–2.129 | −1.01 ± 0.16 | 0.76 ± 0.07 | 0.98 | $P = 0.014$ | NS | $P < 0.001$ |
| | High | Control | 23 | 0.022–7.711 | −0.55 ± 0.13 | 0.73 ± 0.08 | 0.97 | NS | NS | $P < 0.001$ |
| | | Elevated | 16 | 0.149–4.971 | −0.61 ± 0.06 | 0.76 ± 0.06 | 0.99 | $P = 0.012$ | NS | $P < 0.001$ |
| *Ophiothrix fragilis* | Low | Control | 22 | 0.007–0.222 | −1.94 ± 0.19 | 0.69 ± 0.07 | 0.97 | NS | NS | $P < 0.001$ |
| | | Elevated | 22 | 0.011–0.294 | −2.12 ± 0.17 | 0.62 ± 0.06 | 0.97 | NS | $P < 0.001$ | $P < 0.001$ |
| | Medium | Control | 22 | 0.007–0.205 | −1.52 ± 0.27 | 0.63 ± 0.10 | 0.94 | NS | $P = 0.032$ | $P < 0.001$ |
| | | Elevated | 20 | 0.021–0.223 | −1.33 ± 0.44 | 0.71 ± 0.17 | 0.89 | NS | NS | $P = 0.003$ |
| | High | Control | 20 | 0.043–0.222 | −1.31 ± 0.31 | 0.73 ± 0.14 | 0.93 | NS | NS | $P = 0.001$ |
| | | Elevated | 23 | 0.004–0.246 | −1.20 ± 0.23 | 0.72 ± 0.08 | 0.96 | NS | NS | $P < 0.001$ |
| *Amphiura filiformis* | Low | Control | 19 | 0.005–0.035 | −1.70 ± 1.19 | 0.92 ± 0.29 | 0.84 | NS | NS | NS |
| | | Elevated | 22 | 0.002–0.036 | −3.22 ± 0.98 | 0.56 ± 0.23 | 0.73 | NS | NS | $P = 0.001$ |
| | Medium | Control | 23 | 0.010–0.044 | −3.18 ± 1.55 | 0.46 ± 0.38 | 0.46 | NS | NS | $P = 0.012$ |
| | | Elevated | 22 | 0.007–0.050 | −1.71 ± 1.22 | 0.83 ± 0.30 | 0.77 | NS | NS | NS |
| | High | Control | 22 | 0.006–0.039 | −0.31 ± 0.98 | 1.05 ± 0.23 | 0.89 | $P = 0.004$ | $P = 0.021$ | NS |
| | | Elevated | 22 | 0.002–0.030 | −1.95 ± 1.22 | 0.68 ± 0.27 | 0.74 | NS | NS | $P = 0.031$ |

For each treatment; sample size (*n*); mass range (minimum to maximum specimen AFDM in each treatment group); and mass-against-$VO_2$ linear model parameters (±95 % CI) of the form $\ln VO_2 = \ln M * b + \ln a$, where $VO_2$ is oxygen uptake ($mgO_2\ h^{-1}$) and *M* is ash-free dry mass (AFDM, g). Final three columns show tests of slope *b* against commonly proposed 'universal' and boundary values (NS = no significant difference)

dry mass (AFDM). Oxygen consumption was determined for each specimen by averaging the uptake rate over the period in which oxygen was reduced from 95 to 90 % of air-saturated concentrations. This allowed adequate time for the specimen to become accustomed to the experimental apparatus, but without inhibition to $O_2$ uptake due to increasing hypoxia. Values for $VO_2$ and AFDM were log-transformed and linear ordinary least squares (OLS) regression analysis performed on these data. To compare slope (*b)* and elevation of the resulting linear models, analysis of covariance (ANCOVA) was performed with mass as primary variable, and treatment, species identity, temperature, $pCO_2$ level, or combinations thereof as covariates as appropriate. Tank identity was included as a nested factor within treatment, but no tank effects were observed so this was removed from further analysis. Post hoc tests were performed by rerunning each ANCOVA analysis while varying the initial comparison factor(s) and examining the resulting pairwise comparisons between treatment groups. All data met the homogeneity of variance and normality assumptions of the ANCOVA tests (Bartlett and D'Agostino tests, respectively). Regression slope values (*b*) were also tested against commonly proposed standard or boundary values of 0.667, 0.75 and 1.0 (Table 2).

In order to examine changes to metabolic rates at a representative standard mass, the metric $L_m$ was calculated for each treatment group according to the method described by Killen et al. (2010), this being the mass-specific metabolic rate predicted by the linear model at the midpoint mass of the species mass range in log space. Around this point, changes to the value of the slope have minimal effect on predicted elevation along the *y* axis, and allow for comparisons of metabolic rate between treatment groups at a standard mass (Fig. 1). $Q_{10}$ values were also determined at this same log-midpoint mass using the equation $Q_{10} = (L_{mB}/L_{mA})^{10/TB-TA}$, where $L_{mA}$ is the $L_m$ in the higher temperature (TA), and $L_{mB}$ that in the lower temperature (TB). All statistical analyses were implemented in R (R Core Development Team 2013), and slope tests performed using the SMATR package (Warton et al. 2012).

## Results

### Metabolic rates

Temperature was the major factor affecting metabolic rates in the species examined; in all three species metabolism increased with increasing temperature (Fig. 1)

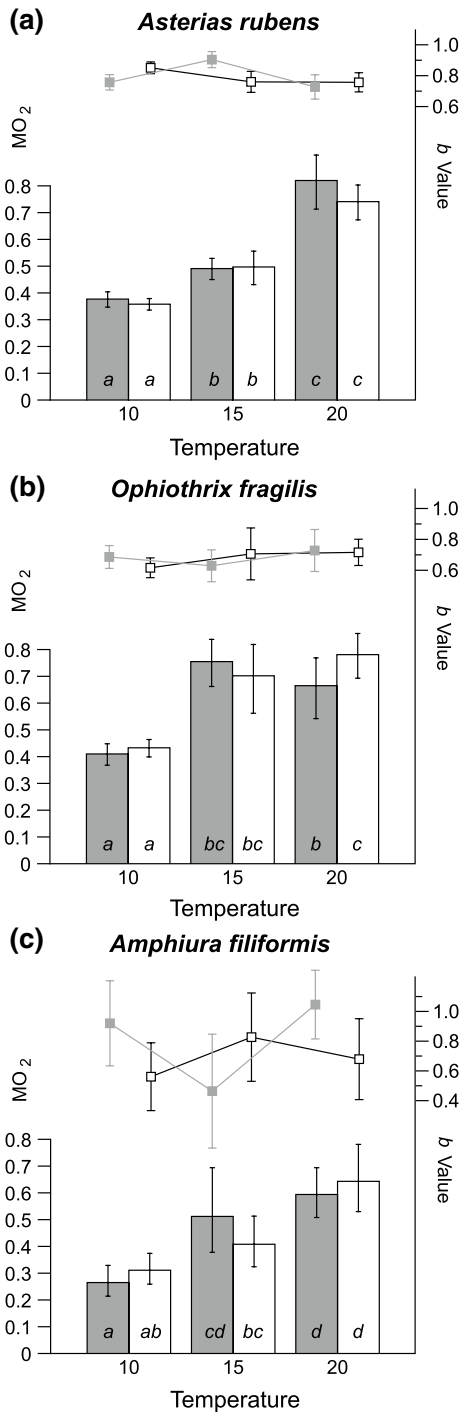


**Fig. 1** Mixed chart showing both $L_m$, the mass-specific oxygen uptake ($MO_2$; $mgO_2\ h^{-1}\ g^{-1}$) at the log-midpoint of the species mass range (*columns, left vertical axis*), and scaling exponent (*b*) values for each treatment linear regression model (*points, right vertical axis*); both ±95 % CI. *Grey* colouring indicates control $pCO_2$ treatments, *white* colouring indicates elevated $pCO_2$ treatments, in each temperature (°C). *Letter codes within bars* denote statistically equivalent $VO_2$ ($mgO_2\ h^{-1}$) between treatment groups (based on ANCOVA results): **a** *Asterias rubens*, **b** *Ophiothrix fragilis*, **c** *Amphiura filiformis*

**Table 3** $Q_{10}$ values for each temperature interval

| Species | $pCO_2$ treatment | Temperature interval | $Q_{10}$ | Mean $Q_{10}$ |
|---|---|---|---|---|
| *Asterias rubens* | Control | Low to Medium | 1.55 | 2.12 |
| | | Medium to High | 2.91 | |
| | | Low to High | 2.04 | |
| | Elevated | Low to Medium | 1.71 | |
| | | Medium to High | 2.30 | |
| | | Low to High | 1.95 | |
| *Ophiothrix fragilis* | Control | Low to Medium | 2.72 | 1.74 |
| | | Medium to High | 0.77 | |
| | | Low to High | 1.56 | |
| | Elevated | Low to Medium | 2.21 | |
| | | Medium to High | 1.25 | |
| | | Low to High | 1.72 | |
| *Amphiura filiformis* | Control | Low to Medium | 2.94 | 2.11 |
| | | Medium to High | 1.36 | |
| | | Low to High | 2.10 | |
| | Elevated | Low to Medium | 1.56 | |
| | | Medium to High | 2.58 | |
| | | Low to High | 1.95 | |

Mean $Q_{10}$ is the average of the four Low-to-Medium and Medium-to-High $Q_{10}$ values for each species. Average temperatures from each treatment (Table 1) were used for calculations; Low 9.4 °C, Medium 15.5 °C, High 20.3 °C

[ANCOVA, $F_{(2,352)} = 270.96$, $P < 0.001$]. However, species-specific responses to the temperature treatments differed. The asteroid *A. rubens* showed a greater increase in metabolic rates between the medium and high temperature treatments than between low and medium (Fig. 1a), as indicated by the higher $Q_{10}$ values in these intervals in both $pCO_2$ treatments (Table 3). The ophiuroid *O. fragilis* showed a strong response to temperature between low and medium treatments (Fig. 1b), as indicated by the high $Q_{10}$ values for these intervals (Table 3). However, there was no further increase in metabolic rate with increasing temperature (Fig. 1b). The low mean $Q_{10}$ of 1.74 in *O. fragilis* was clearly a result of the general equivalence of metabolic rates in medium and high temperatures (Fig. 1b), and when based on only the low-to-medium temperature intervals would be 2.46, within the expected range (Lawrence 1987). The ophiuroid *A. filiformis* showed generally regular increases in metabolic rate in response to increasing temperature across both $pCO_2$ treatments (Fig. 1c).

Across all three species, elevated $pCO_2$ had no apparent effect on metabolism [ANCOVA, $F_{(1,352)} = 0.23$, $P = 0.63$]; no species showed significantly different oxygen uptake in elevated $pCO_2$ treatments compared to

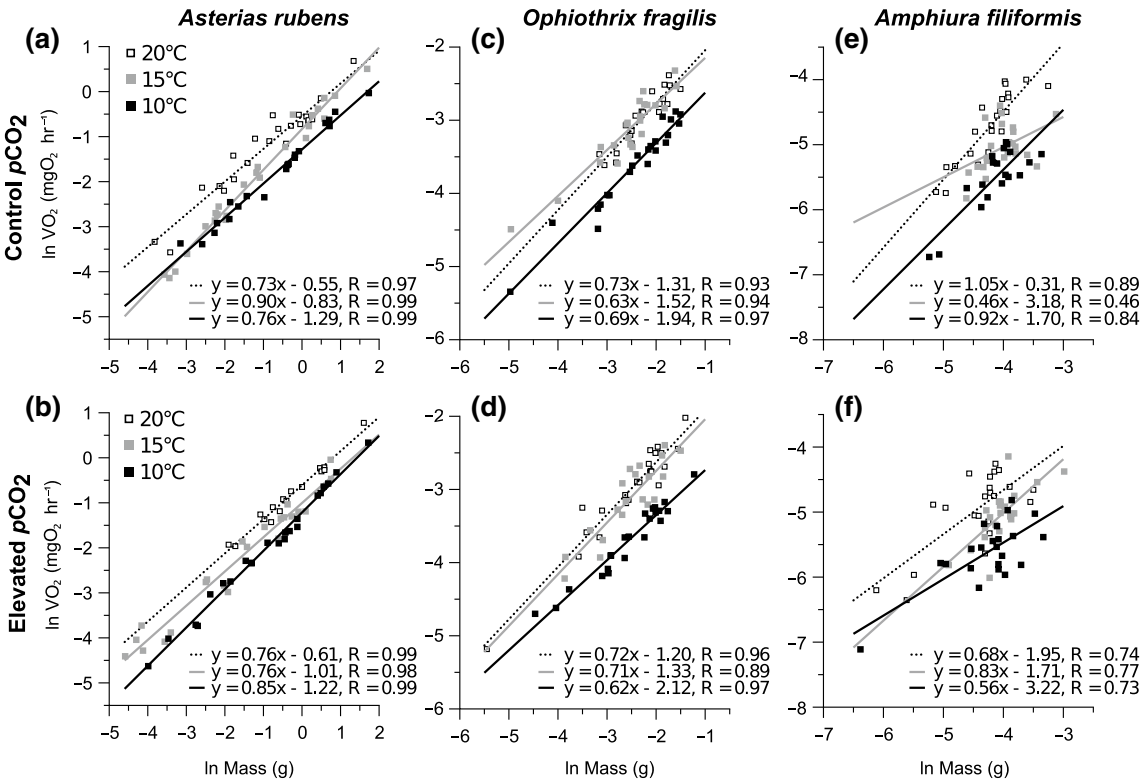


**Fig. 2** Linear models of ln $VO_2$ ($mgO_2$ $h^{-1}$, *vertical axes*) against ln Mass (g, AFDM, *horizontal axes*), separated by species and $pCO_2$ treatment. **a** *A. rubens*, control $pCO_2$; **b** *A. rubens*, elevated $pCO_2$; **c** *O. fragilis*, control $pCO_2$; **d** *O. fragilis*, elevated $pCO_2$; **e** *A. filiformis*, control $pCO_2$; **f** *A. filiformis*, elevated $pCO_2$. In all *plots*: 10 °C, *black lines*, *black squares*; 15 °C, *grey lines*, *grey squares*; 20 °C, *dashed lines*, *open squares*. Top panels are control $pCO_2$, lower panels are elevated $pCO_2$. All regression model parameters are repeated in the figure legends; these data are the same as those presented in Table 2

control $pCO_2$ at any temperature, the one exception being *O. fragilis* in the high-temperature treatments, where there was a significant difference in oxygen uptake across $pCO_2$ treatments [ANCOVA, $F_{(1,39)} = 8.45$, $P = 0.006$]. However, uptake rates in these same two high-temperature treatments were not significantly different to those in either of the medium-temperature treatments (Fig. 1b), and this particular significant difference across $pCO_2$ levels was clearly not part of any overall pattern.

## Scaling of metabolism

Across all species, the metabolic scaling exponent *b* did not significantly vary with either temperature [ANCOVA, $F_{(2,376)} = 0.46$, $P = 0.63$] or $pCO_2$ [ANCOVA, $F_{(1,376)} = 0.63$, $P = 0.43$]. Particular pairwise comparisons of slope across $pCO_2$ or temperature level occasionally showed significant differences in the value of *b* [e.g. in *A. rubens,* across $pCO_2$ treatments in medium temperatures; $F_{(1,43)} = 10.89$, $P = 0.002$]; however, there was no consistent trend in *b* value with either of these factors and, overall, differences were not significant in any species.

In *A. rubens,* the value of *b* differed significantly between temperature treatments [$F_{(2,123)} = 4.39$, $P = 0.014$], however there was no overall trend to these differences. The value of *b* did not differ significantly across $pCO_2$ level [$F_{(1,125)} = 0.05$, $P = 0.83$]. Values lay within and were, with one exception, significantly different to the proposed boundaries of 0.667 and 1.0 of the MLB hypothesis (Table 2). In addition, the majority of *b* values were not significantly different from the canonical 'universal' value of 0.75 (Fig. 2; Table 2).

In *O. fragilis*, the value of *b* did not differ significantly with either temperature [$F_{(2,123)} = 0.47$, $P = 0.63$] or $pCO_2$ [$F_{(1,125)} = 0.02$, $P = 0.89$]. Values of *b* lay close to the MLB boundary value of 0.667, and none were significantly different to this value. The majority were also not significantly different to 0.75 (Fig. 2; Table 2).

In *A. filiformis*, errors around *b* values were widely dispersed and no trend with experimental variables was apparent. Values for *b* did not differ significantly with either temperature [$F_{(2,124)} = 0.69$, $P = 0.51$], or $pCO_2$ [$F_{(1,126)} = 1.13$, $P = 0.29$]. However, *b* values lay approximately within the boundaries proposed under the MLB hypothesis (Table 2). The majority of *b* values were not

significantly different from either of the commonly proposed 'universal' values of 0.667 and 0.75 (Fig. 2; Table 2).

## Discussion

### Effects of temperature and $pCO_2$ on metabolic scaling

Following a lively historical debate over particular values for the metabolic mass scaling exponent (Agutter and Wheatley 2004), recent consensus has recognised that the value of $b$ varies between species, within certain boundaries (Glazier 2005; Kolokotrones et al. 2010; Isaac and Carbone 2010; White 2011; White and Kearney 2013). The data presented here would appear to support the historically accepted view that $b = 0.75$. However, the three species examined here comprise a small contribution to the entire dataset of organismal scaling, and there is ample evidence that scaling exponents show variation between taxa (Glazier 2005; Seibel 2007; Killen et al. 2010; White and Kearney 2013). Metabolic scaling may also vary *within* taxa during different life stages (Gaitán-Espitia et al. 2013; Jensen et al. 2013), and under different abiotic conditions, such as higher temperatures (Killen et al. 2010; Luo and Wang 2012; Ohlberger et al. 2012; Doyle et al. 2012; Weldon et al. 2013).

Our data show that the echinoderms examined here possess a remarkable degree of consistency in metabolic scaling under different physical and chemical seawater conditions (Fig. 1). One aim of this study was to test a specific prediction of the MLB hypothesis (Glazier 2010) that due to the exponential effects of temperature on metabolic rates, the metabolic scaling exponent $b$ will decrease with increasing temperature (Killen et al. 2010; Ohlberger et al. 2012; Gifford et al. 2013). This work also represents the first empirical study testing the direct effects of OA conditions on metabolic scaling.

The values for $b$ found here are consistent with the common range in echinoderms of 0.6–0.8 noted by Lawrence and Lane (1982), although some values in the asteroid *A. rubens* were higher, and some in the ophiuroid *A. filiformis* both higher and lower (but with large error) (Table 2). The general values of $b$ here are consistent with those found for deep-sea echinoderms by Hughes et al. (2011), of 0.90 for Asteroidea (represented here by *A. rubens*), and 0.68 for Ophiuroidea (represented here by *O. fragilis* and *A. filiformis*). Lawrence and Lane (1982, Table 2) also present values that are in general substantially higher in asteroids than in ophiuroids. Although values of $b$ in *A. rubens* and *O. fragilis* were not significantly different from 0.75 (Table 2), values for *A. rubens* were higher than those for *O. fragilis* (with one exception where they were equal) and suggests that these two species possess different species-specific scaling exponent values. This is supported by ANCOVA post hoc pairwise comparisons, where all but the lowest $b$ values in *A. rubens* are significantly different from the values for *O. fragilis*. As such, the differences in the value of $b$ between species observed here, but no apparent effect of the abiotic factors, may reflect taxonomic differences in physiology rather than environmentally-driven changes to intraspecific metabolic patterns.

Scaling exponent values in the other ophiuroid species, *A. filiformis*, had much wider errors than those in the other two species, obscuring any potential trend in the value of $b$ with the experimental treatments. This large error likely comes from the fact that this species is much smaller than the other two (maximum AFDM was 0.05 g compared to 0.29 g in *O. fragilis* and 7.71 g in the asteroid *A. rubens;* Table 2) and reflects the experimental challenges of small body sizes. When body size ranges are small, residual variation has a large influence on the estimates of $b$; mass ranges of two to three orders of magnitude are recommended for providing more robust estimations of metabolic scaling slope (Sokal and Rohlf 1995; Moses et al. 2008; White and Kearney 2014). Here, the mass ranges of *A. filiformis* treatment groups were in general within one order of magnitude (Table 2), likely explaining the wide errors around estimates of the slope in the linear regressions (Fig. 2). This was not an issue with the other two species where mass ranges were two to three orders of magnitude. Figure 2e, f shows that in some *A. filiformis* treatment groups (particularly medium temperatures), regression slope estimates are probably not particularly robust. However, the general elevations of the regression models are clearly higher as a result of increasing temperatures, indicating higher metabolic rates, as confirmed by ANCOVA analysis and comparisons using the $L_m$ metric. Conclusions, however, about the nature of variation in $b$ are difficult to make from these data in *A. filiformis*.

Our results do not support the prediction of the MLB hypothesis that $b$ should depend on temperature. This apparent stability in metabolic scaling under different temperatures conflicts with several other recent studies. Killen et al. (2010) in a large meta-analysis of fish species showed scaling of metabolism (that is, the value of $b$) is inversely related to temperature. A negative effect of increasing temperature on metabolic scaling has occasionally been reported in experimental studies on various ectotherms, including in fish (Hölker 2003; Luo and Wang 2012; Ohlberger et al. 2012), an onychophoran (Weldon et al. 2013), and an amphipod (Doyle et al. 2012). However, the effects of temperature on $b$ can be highly variable between species (Glazier 2005), including positive (Newell 1973), and, as with the present data, no apparent effects in some species (Ohlberger et al. 2012; Gifford et al. 2013).

The MLB hypothesis, however, describes large-scale patterns in physiology, and it is to be expected that limited datasets could produce exceptions and outliers that appear to be inconsistent with its predictions. Ohlberger et al. (2012) found that plasticity in *b* with temperature differed at the family level in the four fish species examined, two species showing a negative association of *b* with temperature, but two others showing no association. But the two groups of fishes represent different habitats as well as systematic groupings, so plasticity of responses to temperature may also be related to species' ecology (Ohlberger et al. 2012). The populations sampled in the present study live in relatively stable thermal conditions. Two species examined here, the asteroid *A. rubens* and the ophiuroid *O. fragilis*, are ubiquitous North Atlantic intertidal and shallow-water coastal species, and the intertidal is a naturally highly variable habitat experiencing large, twice-daily temperature fluxes (Truchot and Duhamel-Jouve 1980). However, the collection site for our experiments, a Swedish fjord, experiences very little tidal height variation, effectively possessing no intertidal zone. The particular specimens of *O. fragilis* examined here were collected from below the pycnocline, which delineates the highly thermally stable bottom waters (5–15 °C annual range) from the more variable surface waters (0–25 °C annual range, with often substantial diurnal changes). *O. fragilis* was the species with the most stable metabolic scaling value across the experimental treatments (Fig. 1). Specimens of *A. rubens* collected from the more thermally variable surface waters, ca. 5–10 m depth showed marginally more variation in *b* value than *O. fragilis* (Fig. 1). These surface waters, while more seasonally thermally variable than those below the pycnocline, are however still stable in comparison with the temperatures experienced in the intertidal or shallow subtidal in other coastal regions where wide temperature extremes can be experienced twice daily. By contrast, Doyle et al. (2012) found a strong negative relationship between temperature and the value of *b* in an Antarctic amphipod, this also being in a region with extremely narrow aquatic thermal variability (~1.6 °C annually, Doyle et al. 2012), but also note this species colonises the intertidal in summer months where it may experience temperatures of up to 8 °C. These patterns of stability in *b* value correlated with low variability in habitat temperature are intriguing, but at present circumstantial. How natural temperature exposure and habitat may affect plasticity in *b* is a promising avenue for future research and may inform on the validity of particular models and theories. Strong patterns in the value of *b* with habitat parameters have previously been observed, such as in cephalopods where *b* appears to decrease with depth (and hence with lifestyle type and activity) (Seibel 2007), and such patterns provide considerable support for the MLB model (Glazier 2010). The lack of response of *b* may also potentially be explained through the temperature range the species were exposed to. While $Q_{10}$ values were typical for ectotherms (Table 3), there was substantial variation in metabolic responses between different temperature treatments (Fig. 1), with both large and minor metabolic changes observed with each 5 °C increase within a species. It is possible that acclimation to a greater temperature differential over a longer time frame would lead to both more consistent responses to temperature, and a detectable effect on the value of *b*.

*A. rubens* and *O. fragilis* appear to have different scaling exponent values, *b* being substantially higher in *A. rubens* (Table 2), and this may provide some support for the MLB hypothesis from these data. Anecdotally, we observed *A. rubens* as being much more active than *O. fragilis*, and it also had much higher mass-specific metabolic rates at equivalent mass and temperature (data not shown). Under the MLB hypothesis (Glazier 2010), *b* shows a positive association with metabolic level and activity. While there is strong empirical support for this pattern in other taxa (Glazier 2006; White et al. 2006; Seibel and Drazen 2007), the results observed here between only two species are intriguing, but not sufficient at present to draw strong conclusions about the relationship between metabolic level and the value of *b* in these echinoderms.

It is also important to consider that allometric relationships may differ under different activity or performance states. Our data examined only resting metabolism; it is possible that substantial differences in allometric scaling of metabolism may become apparent under other states, such as under high activity, or within narrowed aerobic scope and performance under temperature extremes (Pörtner and Farrell 2008).

Like temperature, elevated $pCO_2$ conditions also had no effect on the value of *b*. This is not unexpected, as $pCO_2$ in general had no effect on metabolism in these species; metabolic rates in elevated $pCO_2$ treatments were not different from those in control $pCO_2$ in any of the tested species at any temperature (with one exception). Temperature was by far the most important abiotic factor affecting metabolism.

## Effects of temperature on metabolism

The overall effect of temperature on metabolic rates was similar to that reported for other echinoderms, with $Q_{10}$ values lying broadly around the lower end of the range of 2–3 suggested by Lawrence (1987) as typical of echinoderms (Table 3). The high temperature used here (20 °C) was selected to be outside the typical range experienced by our experimental animals in their natal habitats; our medium temperature (15 °C) is at the upper end of the naturally experienced thermal range (Dupont and Lundve, pers. comm.). Yet scaling relationships remained stable at these

higher temperatures, suggesting no systematic ontogenetic difference in response. In other words, for the body mass to metabolism relationship to maintain the same slope values, metabolism across all body sizes must have changed by a relatively equal amount. Additionally, physiological responses suggest that at least two of these species (the asteroid *A. rubens* and the ophiuroid *A. filiformis*) were not unduly stressed by high temperatures, indicated by the generally regular increases in metabolic rate between temperature treatments (Fig. 1; Table 3), and the absence of stress-induced arm autotomisation in the ophiuroid during experiments. Larvae of *A. rubens* have been shown to be tolerant of temperatures up to 20 °C (Benitez Villalobos et al. 2006). While temperature-driven increases to metabolism and the associated increased energetic requirements may cause trade-offs between physiological processes, responses here suggest that the direct effects of changes to ambient temperatures may not be severe in these two species. All three taxa are ubiquitous along the NE Atlantic coast and are found in warmer waters to the south (Hayward and Ryland 1996), so are in general comfortable in higher environmental temperatures.

### Effects of $pCO_2$ on metabolism

Numerous studies show that responses to high $pCO_2$ are complex and modulated by external factors such as temperature (Byrne 2011; Gianguzza et al. 2014), food supply (Cohen and Holcomb 2009; Hettinger et al. 2013), salinity (Dickinson et al. 2013), or body size (Waldbusser et al. 2010). Organisms in the coastal zone and intertidal may experience frequent pulses of low pH (Christensen et al. 2011; McElroy et al. 2012). Elevated $pCO_2$ has been shown to induce a 'narcotic' effect in some echinoderms at certain environmental temperatures (Byrne 2011; Christensen et al. 2011), likely a specific response by some intertidal organisms to conserve resources during these periodic low hypercapnic events (Fabry et al. 2008; Pörtner and Farrell 2008; Byrne 2011; Christensen et al. 2011). Other invertebrates may instead show elevated metabolic rates at low pH (Wood et al. 2008, 2010; Stumpp et al. 2011, 2012; Catarino et al. 2012) that may be explained through metabolic upregulation to compensate for tissue acidosis (Stumpp et al. 2012), although not all species are capable of this response (Pane and Barry 2007; Hernroth et al. 2011; Dupont and Thorndyke 2012). Two of the species examined here, the asteroid *A. rubens* and the ophiuroid *A. filiformis,* are known to be incapable of compensating for tissue acidosis (Hernroth et al. 2011; Hu et al. 2014).

We did not record a metabolic response to elevated $pCO_2$, either in isolation or in combination with temperature for any of the three study species. Yet previous experiments have suggested that the same species are vulnerable to OA conditions. Long-term exposure (6 months) to OA has been shown to impact the immune response of *A. rubens* (Hernroth et al. 2011), with implications for vulnerability to pathogens in future oceans (Matozzo et al. 2012; Dupont and Thorndyke 2012). Collard et al. (2013) apparently found a significant decrease in metabolic rate in *A. rubens* after 3 weeks acclimation to lower pH; however, this effect appeared marginal. Conversely, Wood et al. (2008) found that metabolism increased in *A. filiformis* under increased $pCO_2$. Our results here support neither of these findings. High $pCO_2$ (though greatly higher than that examined here; ~3,500 ppm) caused severe retardation to growth rates and disturbance to feeding behaviour in *A. rubens* (Appelhans et al. 2012). These studies on *A. rubens* (Appelhans et al. 2012; Collard et al. 2013) concluded, however, that in general this asteroid is robust to near-future conditions of elevated $pCO_2$. Our data (showing no metabolic response of post-larval adults to a greater increase in $pCO_2$) could also be taken as an indicator of the robustness of this species to OA. However, vulnerability at only one life stage could have severe repercussions to the fitness of species (Byrne 2011; Dupont et al. 2012). In the larvae of *O. fragilis*, a moderate increase in $pCO_2$ (a decrease of ~0.2 pH) caused high mortality and increased developmental abnormality (Dupont et al. 2008).

## Conclusions

The evidence presented here that *b* values are stable under different stressors is an indication that any responses to reasonable predictions of near-future environmental change are not body size dependent in these species; responses, if observed, were relatively equivalent across ontogeny. A change in the value of *b* would represent a differential response of metabolism to stressors in different body sizes. The consequence of this is that relative changes to metabolism are preserved across the species size range; the elevation of the regression model is raised (or lowered) equally along its length, thereby maintaining the slope. This is an indication that the physiological effect of the stressor is equal regardless of the size of the animal. In other words, smaller individuals are no more resilient or vulnerable to the stressor than larger individuals. Such stability in responses across size ranges has important implications for the stability of ecological food webs. Size-dependent changes to metabolic rates have been noted as potentially causing cascading effects through trophic levels (Killen et al. 2010; Ohlberger et al. 2012). Potential changes to metabolic scaling are important from an ecological perspective as they may cause altered size dependency in ecological interactions (Ohlberger et al. 2012), or alter

processes which are intrinsically linked to metabolic rates, such as carbon cycling (Kagata and Ohgushi 2012).

While our data show equivalent metabolic responses across the body size range in these species, there is abundant evidence that body size will play a substantial role in determining responses to climate change stressors. Larger individuals may be more sensitive to extreme temperatures (Pörtner and Knust 2007), and species, particularly aquatic ones, may become smaller under climate warming (Sheridan and Bickford 2011; Forster et al. 2012). Body size dependent responses to climate change stressors, such as hypoxia or increases in $pCO_2$ and temperature, have however been observed in other taxa (Killen et al. 2010; Ohlberger et al. 2012), and in other physiological and behavioural phenomena other than metabolism (Waldbusser et al. 2010; Clark et al. 2013). In experimental studies, it is common practice to use a 'standard' or narrow size range of an organism in order to reduce the 'confounding' effect of body size on responses (e.g. Wood et al. 2010; Melatunan et al. 2011). However, body size and potential differences in effects across ontogeny are an important variable in species' responses to climate change and should be not be ignored through the desire to simplify future experimental work.

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