Near-future ocean acidification impacts maintenance costs in sea-urchin larvae: identification of stress factors and tipping points using a DEB modelling approach

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Jager T, Ravagnan E, Dupont S. 2016. Near-future ocean acidification impacts maintenance costs in sea-urchin larvae: Identification of stress factors and tipping points using a DEB modelling approach. Journal of Experimental Marine Biology and Ecology 474:11-17. http://dx.doi.org/10.1016/j.jembe.2015.09.016.

ABSTRACT

Ocean acidification (OA) affects the life-history traits of marine invertebrates. To understand the effects of OA on the life cycle, and to assess its ecological consequences, it is essential to look at bioenergetics. Dynamic Energy Budget (DEB) models are particularly useful to quantitatively assess the effects of (multiple) stressors on all life-history traits in an integrated manner. Here, we apply a simplified DEB model (DEBkiss) to previously published data on growth, feeding and respiration of larval green sea urchins (*Strongylocentrotus droebachiensis*), exposed to a range of pH values. Overall, the standard DEBkiss model provides a good explanation of the larval traits over its development from egg to the maximum larval body size. The observed effects of OA were best explained by the hypothesis that OA increases the maintenance costs of the larvae. This increase in maintenance is reflected in a slower development and increased respiration, but has no negative effects on feeding (when comparing animals of similar body sizes). However, it appears that older larvae are able to compensate to some extent for these increased costs by increased feeding and/or decreasing their maintenance rates. The stress factor on energetic processes (such as maintenance costs) is a useful measure of stress that aids the comparison of species, life stages and stressors, and also allows for multi-stress analyses. Interestingly, the stress factor for OA shows an apparent tipping point around a pH of 7.5, which corresponds to the extreme of the present-day natural variability. This result offers an interesting perspective on the potential relationship between natural variability in pH and species sensitivity to OA.

Keywords: Sea urchins, Larval development, *Strongylocentrotus droebachiensis*, Dynamic Energy Budget, Ocean acidification, DEBkiss

1. Introduction

Since the beginning of the industrial revolution, atmospheric CO_2 concentrations have increased from 280 to 400 ppm and are expected to double by 2100, with well-described consequences for the climate (global warming, increase in extreme events frequency, etc.). The ocean represents a major sink for CO_2 and absorbs half of the excess of this gas. Continued uptake of CO_2 alters the carbonate chemistry of the ocean and increases the concentration of hydrogen ions, thereby reducing pH, a phenomenon called ocean acidification (Caldeira and Wickett, 2003). A growing body of evidence demonstrates that this ocean acidification can impact survival, growth, development and physiology in marine invertebrates (e.g., Wittmann and Pörtner, 2013). Building upon the large body of existing literature, the field of ocean acidification is now moving forward. It was argued that any large scale projection of future impacts requires understanding of the mechanisms in action, how they are intertwined across levels of biological organization (molecular, cellular, systemic, ecosystem, all as a result of evolution), and their potential hierarchies (Dupont and Pörtner, 2013). To increase our predictive power, a mechanistic understanding of the physiological response of organisms exposed to ocean acidification is required (see e.g., Woodin et al., 2013).

Sea urchin larvae are excellent candidates to mechanistically investigate the physiological response to ocean acidification. Sea urchin development has been studied for over a century, and the complex nets of intercellular communications leading to each temporal developmental event and stage are well known. They are also sensitive, simple, and reliable tools for assessing and monitoring marine pollution (e.g., Shaw et al., 2009). Sea urchins were identified early on as a primary target for ocean acidification research, and are today one of the most studied models in this field (Dupont and Thorndyke, 2014). The green sea urchin (*Strongylocentrotus droebachiensis*) is a particularly popular test species; it is widely distributed and plays a key ecological and economical role in boreal coastal ecosystems. When exposed to ocean acidification, the planktotrophic pluteus larvae of the green sea urchin showed a high level of plasticity. Development of normal, although showing morphological plasticity, swimming larvae was possible as low as $pH_T \geq 7.0$. Within that range, decreasing pH increased mortality and asymmetry, and decreased growth rate. Respiration rates increased with decreasing pH suggesting changes in the energy budget. At the lowest pHs ($pH_T \leq 6.5$), development was arrested and no larva survived past 13 days post fertilisation (Dorey et al., 2013). Physiological studies revealed that pluteus larvae are unable to compensate for an extracellular acidosis (pHe) resulting from an exposure to ocean acidification. However, the calcifying primary mesenchyme cells are able to fully compensate an induced intracellular acidosis (pHi) using a bicarbonate buffer mechanism involving secondary active Na+-dependent membrane transport proteins (Stumpp et al., 2012). Additional energetic costs also derived from compensatory mechanisms associated with larval gastric pH changes (Stumpp et al., 2013). It was hypothesized that the associated cost of pHi regulation leads to a shift in energy budget, with less energy available for growth (Dupont and Thorndyke, 2014; Stumpp et al., 2011).

To quantitatively test this hypothesis, and to assess its ecological consequences, it is essential to look at all life-history traits of the sea-urchin larvae over its development in an integrated analysis. Dynamic Energy Budget (DEB) models are particularly useful for such quantitative assessments. DEB theory is one of the most comprehensive framework for bioenergetics (Kooijman, 2001; Nisbet et al., 2000), and models based on this theory have been extensively applied to understand the effects of chemical stress (see Jager and Zimmer, 2012; Jager et al., 2006) and environmental factors such as food and temperature (e.g., Freitas et al., 2009), including the ecological consequences of climate change (e.g., Teal et al., 2012). Recently, Muller and Nisbet (2014) presented a DEB model for the effects of ocean acidification on calcifying phytoplankton (coccolithophores). Here, we apply the simplified DEB-based model DEBkiss (Jager et al., 2013) to previously published data on the effects of ocean acidification on growth, feeding and respiration in sea-urchin larvae.

2. Materials and methods

2.1. Experimental data

Data were taken from the studies published in Dorey et al. (2013), Stumpp et al. (2013) and Chan et al. (2015), which used the same methodologies. Briefly, adult urchins were spawned and gametes fertilized. Embryos were then cultured in replicated 5L culture vessels at a density of 10 embryos per mL. When reaching the pluteus stage, larvae were fed daily with algae (*Rhodomonas* sp.) at a constant concentration of 150 µg C L^{-1}. Larval cultures were monitored regularly over several weeks. On each observation day, a subsample of >10 larvae was taken, photographed with a digital camera mounted on a dissecting microscope, and body length (BL in µm) was determined. Larvae were cultured in pH_T ranging between 6.98 and 8.06 at 9°C. Data on respiration rates were extracted from Dorey et al. (2013), and data on feeding rates from Stumpp et al. (2013).

2.2. Basic model

We depart from the DEBkiss model, as presented in detail in Jager et al. (2013), and see supporting information. This model is a simplification of the standard DEB animal model (Sousa et al., 2010). The main difference with the standard model is that DEBkiss lacks a reserve compartment, which implies that energy assimilated from food is used directly for the energy-requiring processes (growth, maintenance, etc.) without buffering by a reserve. Storage of energy can be included in the form of a reproduction buffer; in the case of sea urchins, the gonads in adults are a typical example of such a reproduction buffer with a dual purpose of collecting energy for spawning events and energy storage to survive periods of food limitation (Russell, 1998). The DEBkiss model specifies growth, reproduction, feeding, and respiration. As we focus on larvae in this study, the storage in the gonads and reproduction will not be discussed here. The energy flows in an individual embryo/larva are schematically shown in Figure 1.

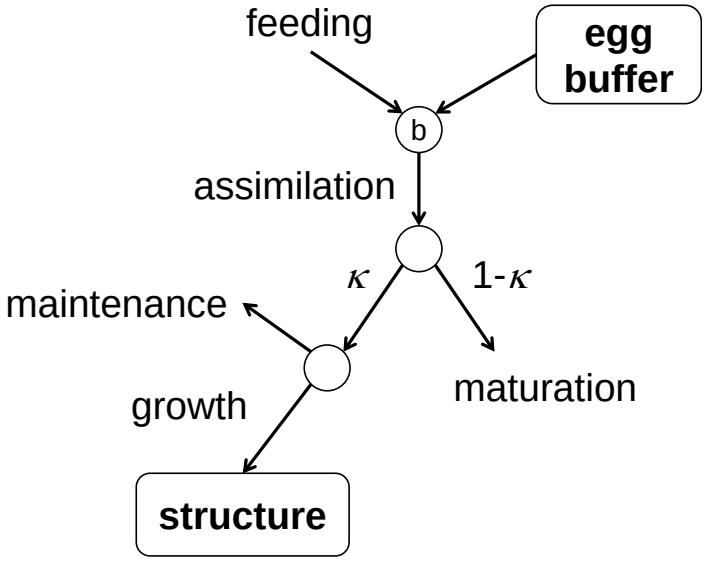

Fig. 1. Schematic representation of the energy flows in embryos and larvae. The node *b* marks 'birth', i.e., the start of external feeding. A constant fraction κ of the assimilation flux is allocated to maintenance and structural growth, where maintenance costs have to be paid first. The 1-κ flux is assumed to dissipate in the maturation process.

In a constant environment, the DEBkiss model for structural growth reduces to the Von Bertalanffy curve. For any well-chosen measure of physical length (L_w, e.g., total body length or test diameter), the differential equation for growth is specified by:

$$\frac{d}{dt}L_w = r_B(fL_{wm} - L_w) \text{ with } L_w(0) = L_{w0} \quad [1]$$

Where r_B is the von Bertalanffy growth rate constant (d^{-1}), L_{wm} is the maximum physical length (mm), L_{w0} the initial length, and f is the scaled functional response (dimensionless; f=1 marks *ad libitum* feeding conditions, and f=0 complete starvation). This equation can be used as long as there are no large changes in body shape with ontogeny, otherwise, a correction is needed (Jager et al., 2015, and see supp. info.). Eq. 1 can be solved to an explicit expression for body length as function of time, as long as all parameters are constant:

$$L_w = fL_{wm} - (fL_{wm} - L_{w0})e^{-r_B t} \quad [2]$$

Within a DEB context, the model parameters r_B and L_{wm} relate to underlying primary parameters of the energy budget (see supp. info.). In DEBkiss (Jager et al., 2013), r_B is related to the volume-specific costs for maintenance (J_M^v in mg/mm^3/d) as follows:

$$r_B = \frac{y_{VA}}{3d_V}J_M^v \quad [3]$$

Where y_{VA} is the yield of structural biomass on assimilated biomass from food (mg dwt/mg dwt), and d_V is the dry-matter density of structural biomass (mg dwt/mm^3). Both these parameters are expected to remain constant with ontogeny, and defaults are often used in practice.

The maximum length (L_{wm}) is determined by the volume-specific maintenance costs (J_M^v), but also by the maximum area-specific assimilation rate (J_{Am}^a in mg/mm^2/d), and the fraction of the assimilated mass that is allocated towards maintenance and growth (κ, dimensionless, see Figure 1):

$$L_{wm} = \frac{\kappa}{\delta_M}\frac{J_{Am}^a}{J_M^v} \quad [4]$$

The shape coefficient δ_M (dimensionless) translates the length measure that was used (e.g., total body length) to volumetric length (the cubic root of biomass volume).

2.3. Effects of stress

In contrast to the DEB model of Muller and Nisbet (2014), we do not include details of the calcification process into our model, but treat it solely as a stress factor on the energetics. Effects of stressors on growth imply a change in the values of the parameters of the energy budget (Jager and Zimmer, 2012; Jager et al., 2006). An effect on each parameter yields a different and specific effect on the growth curve over time. As can be deduced from Eq. 3 and 4, a decrease in the specific assimilation rate affects L_{wm} but not r_B, an increase in the specific maintenance costs affects both L_{wm} and r_B, whereas an effect on the growth yield (y_{VA}) will only affect r_B. To quantitatively assess these effects, we can introduce a stress factor s on the affected parameter. An effect on assimilation can thus be included as a modification of the parameter in the reference situation (indicated by a zero in the subscript):

$$J_{Am}^a = (1 - s)J_{Am0}^a \quad [5]$$

In this way, s=0 implies no stress. An effect on maintenance costs can similarly be included as:

$$J_M^v = (1+s)J_{M0}^v \quad [6]$$

An effect on the growth process can be seen as an increase in the overhead costs for the transformation of assimilates into structural biomass. We can include this as a decrease of the yield as follows:

$$y_{VA} = (1-s)y_{VA0} \quad [7]$$

Without knowing the exact value of the affected parameters, nor the additional conversion factors, we can include the stress factors on the compound parameters r_B and/or L_{wm}. This is possible because the values of the compound parameters are (inversely) proportional to those of the target parameters (see Eq. 3 and 4).

The approach used to include stress of OA on model parameters is quite comparable to that used by Muller and Nisbet (2014). One notable difference with our approach is that Muller and Nisbet use a specific relationship to link the stress factor to pH, whereas we will fit *s* for all treatments and deduce the relationship from the data. Furthermore, Muller and Nisbet include stress at three processes simultaneously (whereas we depart from the assumption that a single process is affected), and use a slightly different formulation for stress decreasing the value of a parameter (division by 1+*s* instead of multiplication by 1-*s*).

2.4. Other endpoints

When all model parameters have a realistic value, they can subsequently be used to predict feeding and respiration rates (see e.g., Jager and Ravagnan, 2015). For a fully quantitative analysis, we need estimates for the additional parameters and conversions mentioned above (κ, δ_M, d_V, y_{VA}). Further, we would also need to convert mg of assimilates obtained from food or lost in respiration to the measured quantities: mg of carbon consumed and pmol of oxygen used. Here, we will restrict the analysis of these traits to a semi-quantitative one; model derivations are provided in supp. info. In short, feeding rate (J_X) is predicted to be proportional to body length squared:

$$J_X = aL_w^2 \quad [8]$$

Where *a* is a proportionality constant, absorbing primary energy-budget parameters and conversion factors (see supp. info.). An effect on maintenance or growth costs is not expected to have any effect on the feeding rate (when comparing animals of the same size). An effect on assimilation might be associated with a negative effect on the feeding rate (and thus a lower value of *a*), but it might also result from an effect on the assimilation efficiency. An effect on the assimilation efficiency would be difficult to confirm in practice, as it would involve the collection and analysis of faecal matter.

The respiration rate is expected to scale with a weighted sum of body length squared and body length cubed. The underlying reason is that respiration has contributions from different processes: maintenance, maturation and overhead costs for growth (and for feeding organisms also the overhead costs for feeding and assimilation). We can thus represent the respiration flux (J_D) as:

$$J_D = bL_w^2 + cL_w^3 \quad [9]$$

Where *b* and *c* are proportionality constants, absorbing primary energy-budget parameters and conversion factors (see supp. info.). An effect on assimilation should lead to a decrease in *b*, but no effect on *c* (and thus overall a lower respiration rate for animals of the same size). An increase in maintenance costs should lead to an increase in *c* but no effect on *b* (and thus an increase in

respiration for the same size). A decrease in growth yield leads to an increase in *b*, and a decrease in *c* (the net result depending on the precise value of the model parameters and conversion factors).

2.5. Model fitting

All model fits are made in Matlab, using means of treatment groups or replicates. We assumed a normal distribution for the residuals, and residuals are weighted according to the number of individual measurement in each mean. Confidence intervals are generated by profiling the likelihood function. Details on the statistical procedure can be found in Jager and Zimmer (2012).

3. Results and discussion

More detailed versions of the plots can be found in the supporting information.

3.1. Mechanism of action: effect on larval growth

We first focussed on the most comprehensive dataset (Dorey et al., 2013), and pooled all data per treatment, to interpret the general pattern in the data and demonstrate the effects of assuming different modes of action. The lowest pH used in this study (6.5) was ignored, as the embryos did not develop. We fit Eq. 2 with a different stress factor for each treatment. The highest pH treatment (nominal value 8.1) is viewed as the reference situation, and hence the stress level is set to zero. Figure 2 shows the model fit to the pooled data for the three single modes of actions specified in the methods section: assuming that ocean acidification (OA) affects the assimilation process (Eq. 5), the costs for maintenance (Eq. 6), or the costs for growth (Eq. 7).

The growth data by themselves do not provide support for selecting any of these single mechanisms of action, and therefore also not for a combination of mechanisms (which would show a response pattern in between these three extremes). Costs for growth generally provides better fit to the data for t>5 days, as it captures the rather similar final size in all treatments. The data points at t=24 days are, however, less certain than the other data points as the number of individuals is much lower (due to mortality and experimental limitations). Assuming an effect on maintenance or assimilation provides a good fit up to t=15 days, but cannot capture the apparent recovery in the highest treatment.

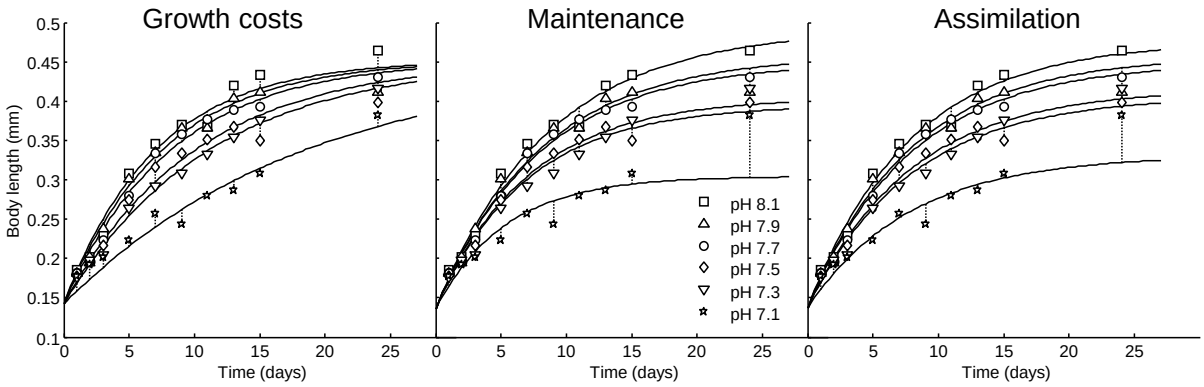

Fig. 2. Growth of sea-urchin larvae under different pH conditions. Time is days post fertilisation. Model fits assuming that OA affects the costs for growth, the costs for maintenance, or the assimilation process. Data from Dorey et al. (2013).

It should be noted that the growth model of Eq. 2 only holds when the organism does not change in shape with ontogeny. For the sea urchin, this assumption is violated, especially in the initial part of

the growth curve. Over the first 5 days post-fertilization, a fertilised egg develops into an embryo through successive cleavages and complex embryogenesis. After hatching (blastula stage), the embryo initiates gastrulation and maturation into a pluteus larvae. These larvae keep evolving through time, growing extra pairs of arms and organs, including morphogenesis of the juvenile rudiment (Smith et al., 2008). If we can establish the relationship between body length and structural biovolume for each stage, we should compensate for these changes (see Jager et al., 2015). In the absence of more detailed biometric information for sea urchin larvae and embryos, this cannot be included in the current analysis.

3.2. Mechanism of action: feeding and respiration rates

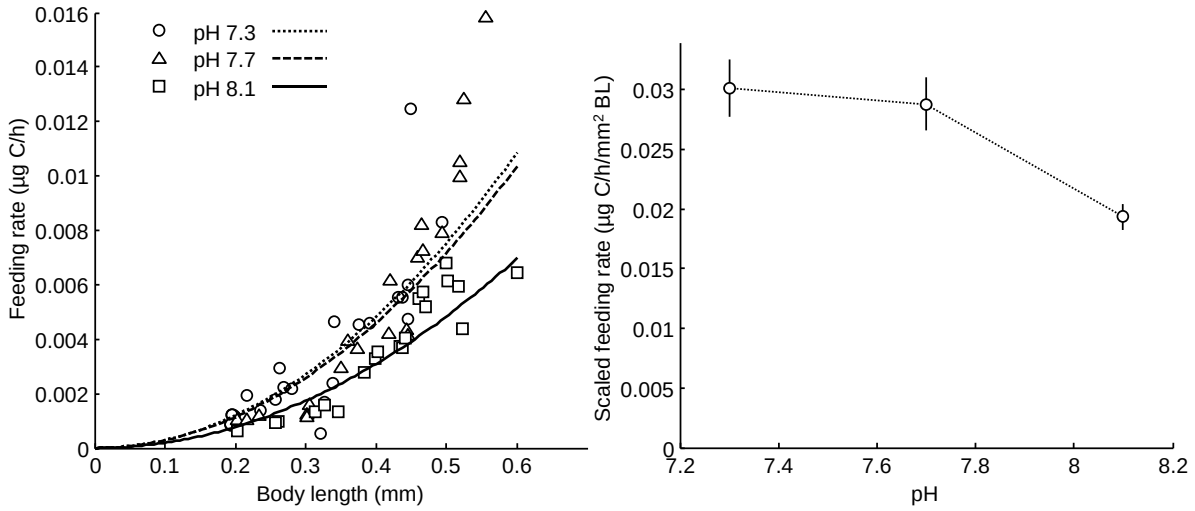

Fig. 3. Left panel: feeding rates of larvae from Stumpp et al. (2013) versus body length. Model lines are the square-root relationships from Eq. 8, where proportionality *a* is taken as the mean value of *a* within a treatment. Right panel: feeding rates, scaled with body length squared (proportionality *a*), versus the nominal pH in each treatment. Bars represent standard error of the mean.

Feeding rate follows the expected relationship to body length squared (Fig. 3, Eq. 8). At low pH, higher feeding rates were observed, which was explained as a compensatory response for the lower digestion efficiency at low pH (Stumpp et al., 2013). Slower growth at low pH values is thus not related to diminished feeding rates, although a net decrease of assimilation due to a stronger effect on assimilation efficiency cannot be excluded.

The respiration rate is more difficult to interpret, as respiration has contributions from different processes such as maintenance, growth overheads, and maturation. In theory, the respiration rate should be proportional to a weighted sum of body length squared and body length cubed (Eq. 9). In practice, however, the respiration measurements were done without food for 14-24 hours (Dorey et al., 2013), making it likely that growth and maturation were reduced. For the feeding stages, we therefore assume that the respiration measurement represents maintenance costs only, and thus scales with body length cubed (setting *b* in Eq. 9 to zero). The measurements at *t*=3 days (non-feeding stages) were excluded from this analysis; they are generally higher than expected from the cubed relationships to body length of the later stages. This likely reflects a continuation of growth and maturation during the measurement, as their metabolism is fuelled by resources provided by the mother in the egg (see Fig. 1). With these assumptions, we obtain a reasonable correspondence to the measured data (Fig. 4), although the scatter is considerable. Plotting the proportionality *c* (in Eq. 9) versus the nominal pH in each treatment gives a clear increase in volume-specific respiration rate with decreasing pH. This makes it unlikely that OA acts through impacts on the assimilation or the growth process; for these mechanisms, no such increase in respiration should be observed.

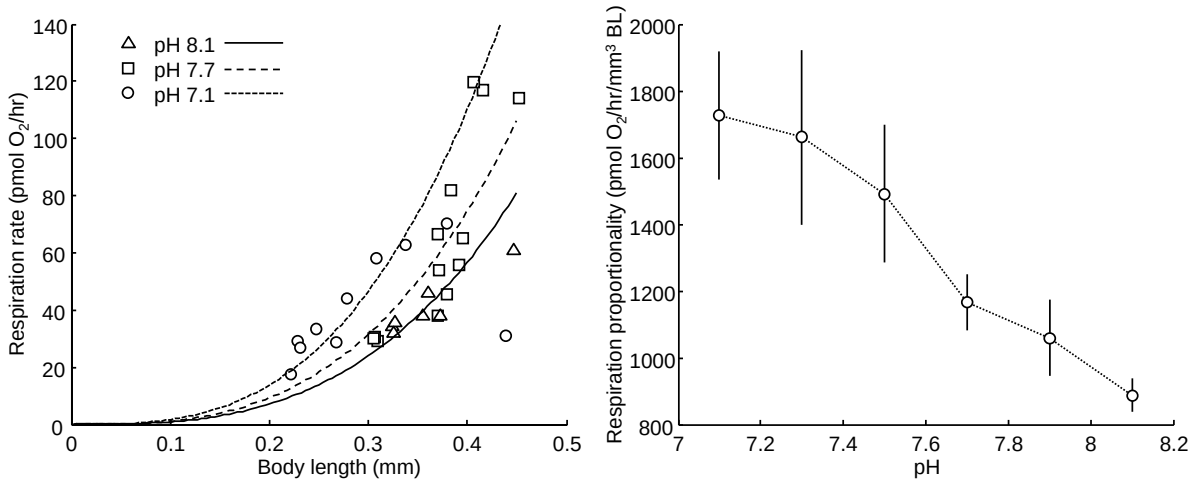

Fig. 4. Left panel: respiration rates versus body length from Dorey et al. (2013) for three pH treatments (all treatments shown in supp. info.). Model lines are the cubed-root relation of Eq. 9 (proportionality *b* set to zero, and *c* taken as the mean value of *c* within each treatment). Right panel: respiration rate, scaled with body length cubed (proportionality *c*), versus the nominal pH in each treatment. Bars represent standard error of the mean.

The proportionality constants *a* (Eq. 8 and right panel of Fig. 3) and *c* (Eq. 9 and right panel of Fig. 4) are quantitatively related to the parameters fitted on the growth curve (Table 1): clearly, the feeding rate needs to be sufficiently large to fuel the energy-requiring processes of growth and respiration. The theoretical relationship between these compound parameters is worked out in the supporting information, but testing the mass balancing requires information that we could not establish for the sea-urchin larvae (especially on the link between body length and carbon content in biomass).

Taken together, the evidence suggests that OA mainly acts by increasing maintenance costs for the sea-urchin larvae. The growth data (see more detailed plots in supp. info.), however, indicate that after t=15 days, the remaining larvae in several of the replicate treatments are able to overcome the stress, and are able to achieve a body length that is close to that of the reference. This may be related to additional compensatory feeding (see the large values for the feeding rate at the largest body sizes, for the low pH treatments, in Fig. 3), and/or to a decrease in the maintenance costs (see the low value for the respiration rate for the largest body sizes in some treatments, in Fig. 4 and supp. info.). However, more detailed investigations are needed to elucidate the mechanisms underlying this compensatory effect.

3.3. Deriving the stress factor

Now that we decided on the most likely mechanism of action, we can study the available growth data in more detail, and derive a stress factor for each replicate treatment. For each replicated aquarium, an average pH was calculated (Chan et al., 2015; Dorey et al., 2013; Stumpp et al., 2013). We fitted each individual replicate with its own stress factor (stress was set to zero for one arbitrary replicate in the pH 8.1 treatment). The basic model parameters (L_{w0}, L_{wm}, r_B) were also fitted, but were forced to the same value for each replicate within a study (see Table 1). We did this analysis for maintenance costs only, and excluded the information from the treatments for t>15 days (for the reference treatments, all points are included). We excluded the data after 15 days from the fit as we do not wish to speculate about additional mechanisms to explain the apparent compensation of the stress response.

The resulting stress factors from the growth data show a strong increase with decreasing pH (Fig. 5). Stress levels are low down to a pH of around 7.6. At lower pH values, the stress increases more steeply with decreasing pH. At the lowest tested pH values, the stress has reached a value of close to one, which implies that the maintenance costs are predicted to be almost a factor of two higher than in the reference (see Eq. 6). This is well in line with the maximum factor of two stress as derived from the respiration rates, although the relationship with pH is different in both cases (Fig. 5). At intermediate pH values, the stress factor from the respiration data is larger than the factor derived from the growth data, whereas we would expect both to have the same value. The large uncertainties in the respiration rates, however, preclude firm conclusion (Fig. 4), but it is certainly possible that the underlying mechanisms are more complex than we assumed.

Seawater pH varies considerably in coastal environment and the present range of variability in the green urchin sampling site is between pH_T 8.7 to 7.6 (Dorey et al., 2013). Previous studies hypothesized that the tolerance range of a given species to decreased pH shall correspond to their natural range of exposure. Indeed, it was demonstrated that different evolutionary (Thor and Dupont, 2015) and physiological mechanisms (Dorey et al., 2013) were involved when tested pH was deviating from the present range of variability. However, using larval growth rates of the green sea urchin, Dorey et al. (2013) estimated the physiological tipping point at pH_T=7.3. Where tipping point was defined by the authors as "the lowest pH limit within which normal development, growth and survival are possible." In practice, the threshold was taken as the pH below which the growth rate revealed a much sharper decrease with decreasing pH. In our new analysis using a DEB modelling approach (Fig. 5), we can similarly identify such a stress tipping point around pH_T=7.5. This more conservative value corresponds to the extreme of the present natural variability and supports our hypothesis. This approach should now be tested on other species and stages of development. If confirmed, this relationship between present pH envelope and species sensitivity may be key to bridge chemical changes to biological sensitivity and allow large scale projections of biological impacts. However, additional research is needed to understand the mechanism behind the relationship between pH and stress factor so we can develop a more quantitative definition of the tipping point.

Interestingly, the data of Stumpp et al. (2013) show a higher stress level around pH=7.3 than the data sets of Dorey et al. (2013) and Chan et al. (2015); the latter two being in close correspondence. The data of Stumpp et al. (2013) also yielded quite different parameter estimates from the other two studies (Table 1). These differences are not model artefacts, but clear differences between experiments: the animals in Stumpp et al. (2013) are smaller than those in the other studies for t<10 days, and larger for t>20 days, although the scatter in this latter part is considerable (see plot in supp. info.). Egg size is highly variable in sea urchins (Scheibling and Hatcher, 2007), and it is thus likely that the eggs from the batch that started the experiment of Stumpp et al. (2013) were smaller than those of the other two studies, and that the smaller egg size was associated with different energetic parameters of the embryos and larvae. A smaller r_B and larger L_{wm} (Table 1) is consistent with a lower value for the maintenance costs (see Eq. 3 and 4). The decrease in r_B is larger than the increase in L_{wm}, (a factor of 1.6 vs. 1.3). However, when fitting all three reference data sets simultaneously, they can be well described by assuming that the animals from Stumpp et al. (2013) only differ in initial size and maintenance costs from the animals in the other two studies (see fits in supp. info).

This highlights the critical importance of considering intraspecific variability when investigating the impact of OA on a given species. Sunday et al. (2011) demonstrated experimentally that maternal effects were playing a key role in determining larval size and response to decreased pH. It was hypothesised that egg provisioning contributes additively to the OA effect. In other words, maternal provisioning could partly buffer against, but not fully mitigate, negative effects of OA. This is consistent with our observations, although the mechanistic links between egg size, maintenance costs and stress due to OA remain to be elucidated. Future work should therefore investigate the impact of OA on egg

quality and quantity, as well as the potential of egg size to identify individuals or populations with stronger potential to cope with OA.

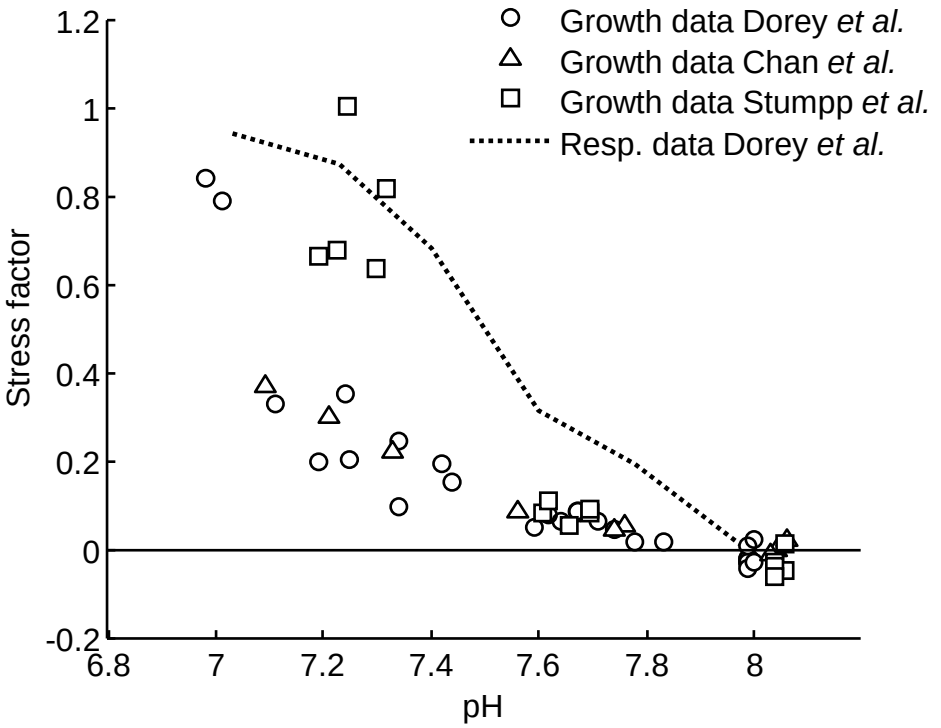

Fig. 5. Stress on maintenance as a function of measured pH. For one of the highest pH treatments in each study, the stress is set to zero. Underlying fits on the replicated growth data are provided in supp. info. Broken line represents the stress factor derived from the respiration data in Fig. 4, plotted against the mean measured pH.

Table 1
Parameter estimates, with 95% likelihood-based confidence interval, for the fits on the individual replicate growth data (fits shown in supp. info.).

Symbol	Parameter	Dorey et al. (2013)	Stumpp et al. (2013)	Chan et al. (2015)
L_{w0}	Initial body length (mm)	0.14 (0.13-0.14)	0.069 (0.061-0.078)	0.13 (0.11-0.14)
L_{wm}	Maximum body length (mm)	0.48 (0.46-0.51)	0.62 (0.59-0.66)	0.50 (0.47-0.53)
r_B	Von Bertalanffy rate constant (d^{-1})	0.12 (0.11-0.13)	0.076 (0.067-0.084)	0.14 (0.12-0.16)
s	Stress factor (-) fitted for each replicate (Fig. 5)	0-0.84	0-1.0	0-0.37

3.4. Flexibility in the maintenance costs

In general, maintenance costs within a species are not expected to vary, apart from the potential effect of stressors like OA that may increase these costs. The data for sea-urchin larvae, however, offer some tantalising clues to a more flexible view of maintenance. The difference in growth pattern in the reference treatments for the studies of Dorey et al. (2013) and Chan et al. (2015) on the one hand, and those of Stumpp et al. (2013) on the other, can largely be explained by a smaller egg size and lower maintenance needs in the latter study. Furthermore, as discussed, the negative effects of OA on growth may have been partly compensated, later in larval development, by reducing basal maintenance needs. At first glance, such flexibility in maintenance costs does not seem to make much sense in an evolutionary context: if maintenance is to some extent optional, an individual with reduced costs would have an advantage and out-compete its more energy-expensive conspecifics. There is, however, the speculative hypothesis of 'waste to hurry' (Kooijman, 2013) that can offer an

explanation. Small organisms that rely on blooming resources (such as phytoplankton) are best able to exploit such a resource if they grow fast and stay small. The only way to do this in DEB theory is to boost the maintenance costs, and hence, wasting resources (i.e., burning assimilated food without useful work). A consequence of this wastefulness is that maintenance needs may be decreased under some conditions without negative effects on the structural integrity of the animal. Hatching from a small egg or suffering from OA might represent such conditions. Admittedly, this explanation for the observed patterns is still highly speculative, but its consistency with the observed patterns in intriguing and requires further dedicated research.

5. Conclusions

The simple DEB analysis applied here helped to interpret the available experimental data in an integrated manner. The model framework makes strong predictions for the patterns of the various traits (growth, feeding and respiration), and these predictions were largely consistent with the observations. Here, we performed a semi-quantitative integration for the different traits, as we lack the detailed information to parameterise a closed mass balance (which needs values for the additional parameters in Eq. 3 and 4, and some other conversion factors).

OA affects maintenance costs in sea-urchin larvae. This explanation is consistent with the slower growth, increased respiration, and lack of negative effects on feeding (when comparing animals of the same size). For the first part of the data set (up to 15 days post fertilisation), this mechanism of action provided an excellent explanation of the observed effect patterns on growth. A clearly pH-dependent increase in stress is shown with increasing acidification, including an apparent tipping point around a pH of 7.5. This result offers an interesting perspective on the potential relationship between present natural variability in pH and species sensitivity to OA. If confirmed in other taxa, this would constitute a powerful tool to link chemical and biological changes in the future ocean. Later in the larval development, it appears that animals were able to compensate to some extent for the stress of OA, at least in some of the replicates. This compensation, and the differences in growth at reference pH between the studies, point at the possibility that the maintenance costs are to some extent flexible. Dedicated research (e.g., more detailed respiration measurements) is needed to support this hypothesis, as well as its ecological consequences.

Here, we only considered the embryonic and larval stages of the sea urchin. After metamorphosis, sea urchins obviously grow to a much larger size than the maximum length established for larvae in Table 1. The conclusion thus needs to be that one or more of the energy-budget parameters changes with metamorphosis. Furthermore, it needs to be investigated whether juvenile/adult sea urchins are affected in the same way, and to the same extent, as the larvae. The DEBkiss framework offers a straightforward platform for such comparisons, as it is more meaningful to compare stress factors on energetic processes (such as maintenance costs) than on life-history traits (such as growth rate) for such different life stages. This way to quantify stress thus makes it easier to compare species, life stages, and stressors, but also allows different stressors and/or environmental factors to be combined in a multi-stress approach (see Jager et al., 2010).

Even though the results of the DEB-based analysis are in line with the conclusions drawn in the original studies, the modelling effort provides additional insight into the underlying mechanisms. Furthermore, it raises more fundamental questions about sea-urchin biology as well as the effects of OA, which can provide a solid basis for further research.

Acknowledgements

We gratefully acknowledge funding from the Research Council of Norway through the project OAPPI (215589), and thank the project leader Renée Bechmann for her support. SD is funded by the Linnaeus Centre for Marine Evolutionary Biology at the University of Gothenburg and supported by a Linnaeus grant from the Swedish Research Councils VR and Formas.

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