We are grateful for the constructive comments from our reviewers. We have made all the necessary comments (blue) to the reviewers queries (italics) and made the necessary changes to our manuscript (red). A strikeout through a reference refers to a figure, table or equation in the original manuscript.

Comment 1 - anonymous reviewer

*My main concern is that the work is a somewhat incremental addition to the literature. It relies extensively on the publication by Ragazzola et al. 2012, both with regard to the data and the general concept. While an expansion to more complex models is valuable, it largely reflects the findings of the 2 vs. 3D structural FE analyses from other fields as cited in the text.*

Ragazzola et al (2012)’s work was highly innovative, however these 2D models were simplistic in their very nature, and the results were not ground truthed against more complex natural geometries. The paper we present here represents novel work because it incorporates three-dimensional geometric models of coralline algae but, importantly, presents for the first time an FE-model of coralline algae derived from µCT data. To our knowledge this is one of the most complex computational models of a marine calcifier. Our results don’t entirely reflect the findings of the 2D model. They show that: 1) a 2D model, such as Ragazzola et al. (2012) cannot accurately represent the mechanical performance of a 3D biological structure; 2) a 3D geometric model (constructed appropriately) can be used to accurately represent the mechanical performance of a biological structure; 3) future climate change may have significant impact on the mechanical performance of these organisms and 4) by verifying the use of simplified models, we can look at species specific changes and tailor our models easily, and accordingly, requiring less user and computing time and power. In response to further reviewers comments, we have performed additional analysis on our models (Page 4), which will add another feature to this paper independent of Ragazzola et al. (2012)’s work.

Page 5 line 91
These highly innovative models however were simplistic in nature. Importantly, they had not been tested to assess if they were a fair representation of skeletal mechanical performance. Consequently, the simple 2D model may have overestimated the distribution and magnitude of stress and hence future vulnerability of algal communities. Here we have developed a set of 3D FE geometric models to represent different aspects of coralline algae morphology and compared these models with a more biologically accurate 3D FE-model generated from computed tomography (CT) data, allowing us to assess the trade-off between computing time (Andersen and Jones, 2006; Romeed et al., 2006) and the need for an appropriate representation of the structure.

Page 15 line 286
As responses to climate change are species-specific, we are therefore able to create models tailor made to individual species and analyse how they react to future climate change.
I found the discussion of the factors not accounted for in the model quite useful (e.g., Mg content, proteins), but to have a larger impact on the readership of BGD, it would be helpful and necessary to expand on the environmental context of these simulations further. E.g., are the changes simulated environmentally significant? How does the load tested relate to typical stresses experienced in situ?

The loads applied to our models were estimated by Ragazzola et al. (2012). Further work (outside the scope of this paper) is required to analyse the nature of loads experienced in situ. We present a first attempt at this here, in additional models with revised loading conditions (see response to reviewer 2).

Page 5 line 86
Although the changes in growth rate were not significant, specimens grown under CO2 conditions predicted for the year 2050 were found to have significantly larger cells and thinner cell walls. These ultrastructure changes resulted in predicted increased vulnerability to fracture compared to present day structures (Fig. 2a-b) as observed in the 2D FE-model.

Page 8 Line 154
In keeping with Ragazzola et al. (2012), a load pressure of 20,000Pa was applied to the top left corner, 40 µm along the external top surface and 40 µm down the left surface of the models. Constraints were applied to the whole bottom surface and on the right hand surface, opposite the loads, 40 µm up from the constrained bottom. This simulated the attachment of the structure to the rest of the thallus (Fig. 4). Even though the same loads as Ragazzola et al. (2012) were used, it is known that the primary hydrodynamic force exerted on marine macroalgae is drag force (Carrington, 1990). Drag (F_{drag}) force can be calculated using equation 1.

\[ F_{drag} = \frac{1}{2}(\rho U^2 A C_d) \] (1)

Where \( \rho \) is the seawater density (approximately 1025 kg m\(^{-3}\)); and \( U \) is water velocity. Subtidal marine macroalgae experience a water velocity on the order of magnitude of 1 m s\(^{-1}\) (Carrington, 1990), while intertidal species can experience breaking waves of up to 25 m s\(^{-1}\) (Denny et al., 2003). A is the algal planform area; and \( C_d \) the drag coefficient (dimensionless index of shape change and reconfiguration of flexible fronds (Carrington, 1990; Dudgeon and Johnson, 1992; Gaylord et al., 1994)). However, there are no data published for resulting in breakage of rhodoliths. The existing literature focused on flexible macroalgae, making it difficult to find loads that are environmentally significant whilst also being species related.
I am not convinced that statements such as we have confirmed previous results that future climate change will lead to a loss in the structural integrity of coralline algae are justified. Yes, the simulations here match the trends of published simulations, though the effect of increasing CO2 is much less, in particular for the strain energy.

We agree with the reviewer that this may be an overstatement, and hence the wording has been changed. See below for our change in manuscript.

Page 15, line 281
Using these more biologically accurate models, we have further supported previous results that state future climate change will lead to a loss in the structural integrity of coralline algae.

But more importantly, there is no validation of simulation results of actual structural damage with field data, and it is not obvious that effects from other adaptations can be ruled out completely.

In regards to validation of simulation results, there is currently no published experimental data on structural damage in coralline algae - in the field or in the lab. Such a study is beyond the scope of this paper. It is known that Mg concentration affects the Young’s modulus of the skeletal tissues. To account for this we conducted a material property sensitivity test (page 11), which highlighted the fact that any changes in Mg concentration, due to environmental changes would not have an effect on the stress results, but strain will be affected.

With the current focus on the simulations alone, it would also be helpful to provide more information on the modelling itself. There is no information given on what is calculated (governing equations or concise citations). Thus, a reader interested in the topic but not familiar with the analysis is not well served.

To accommodate the suggestion we have amended the manuscript.

Page 4 line 81
FEA works by transforming a continuous structure into a discrete number of elements which are connected to each other via nodes. The combination of elements and the interconnecting nodes forms the mesh. Appropriate material properties (Young’s modulus and Poisson’s ratio) are assigned to the elements to mimic the elasticity of the structure. Adequate boundary conditions (magnitude and direction of loading and constraints) are applied and then nodal displacements are calculated in response to the applied boundary conditions and material properties of the model. The nodal displacement is used to calculate the strain and subsequently stress (using the Young’s modulus, see equation 2) and hence mechanical performance of complex structures can be inferred. (For mathematical equations see Mathematics of FEA, (Rayfield, 2007) supplementary material)

\[ E \text{ (Young’s modulus)} = \frac{\sigma \text{ (stress)}}{\epsilon \text{ (Strain)}} \] (2)
Comment 2 - Chris Evenhuis

An interesting choice has been made in the loadings. The loading is asymmetric as the forces are applied to the top-left corner over 40 µm strips while the opposing forces are from the bottom-left over a 40 µm strip on the side but the whole bottom surface. This results the loading being mostly compressive down the diagonal of the cube, but with some shear along the x axis. What is the ratio of the compressive to shear forces? Is this mixing of compressive and shear forces intentional? I would imagine that main impact on the structures from wave motion would be large scale shear stresses rather than compressive ones (the fractures in the picture are lateral which suggests they are more susceptible shear forces). Similarly, I would anticipate that borers would also predominately exert shear stresses, just on a much smaller scale. It may be more instructive to change how the applied and constraint forces so that it better reflects the geometry of the natural environment. Maybe setting up two sets of loadings would be a good way to investigate the relative effects of shear and compressive loads.

The mixing of compressive and shear forces were done due to recreating the same loading environment as Ragazzola et al. (2012). As pressure is applied to the top left corner, there will be a mixture of both compressive stress as well as shear stress. Constraining the bottom surface of the cube and the right hand side, and having the load occurring in the top left hand corner, simulated the edge of the thallus receiving the load. Additionally, we are now including simulations distinguishing load types to include only shear and compressive loads instigated by the comments of the reviewer (see below).

Page 5 line 98
The loading and constraint taken from Ragazzola et al. (2012) were a mixture of shear and compressive forces, which simulated boring forces by an organism exerted on the exposed corner of an attached thallus. As it was assumed that these organisms were more prone to shear forces than compressive ones, we also assessed the effect of sole compressive or sole shear forces on the compartmentalised and the biologically realistic model.

Page 9 line 181
2.1.4. Comparison between shear and compressive loads
The biologically realistic and the compartmentalised model were exposed to different loading scenarios in Abaqus. This included the original load setup explained earlier in section 2.1.3 (Fig. 1a); the compressive loads, where the load was applied to the top of the cube opposite the constraint (Fig. 1b); and shear loads, where the load was applied on the face adjacent to the bottom constraint (Fig. 1c).

As this part of the study moved on from the initial research of Ragazzola et al. (2012), it was decided to use loads defined experimentally based on real wave velocities. Starko et al. (2015) used wave velocities of up to 3.5 m s⁻¹ to assess the effect of branching in flexible wave swept macroalgae, in which they also measured the drag force. Water velocity experienced by subtidal marine macroalgae is on the order of magnitude of 1 m s⁻¹ (Carrington, 1990).
Hence, we used a drag force (0.9N) corresponding to a wave velocity of 3.5 m s$^{-1}$ experienced by a heavily branched macroalgae (similar in branching to our rhodoliths) to carry out our load type comparison tests. Here we have kept the force per unit area constant in order to compare the compartmentalised model to the biologically realistic model. Strain energy is dependent on volume, therefore in order to compare the total strain energy between the models we had to take into account the difference in calcite volume between the biologically realistic and the compartmentalised model (using equation 1) (Dumont et al., 2009).

Using these more environmentally significant forces, we can see that the stresses and strains exerted by these organisms were not as large as those taken from Ragazzola et al. (2012). Accounting for the change in units, the differences between the von Mises stress results are on the order of magnitude of $10^9$ instead of $10^{11}$.

In the biologically realistic model, under the original load setup, stress dissipated throughout the model from the corner where the load was applied to the constrained corner (Fig. 1a). While under the compressive load setup, the stress had a top to bottom distribution (from the loaded surface to the constrained surface) with a slight increase in stress surrounding the cavities in the model (Fig. 1b) and under the shear load setup, two thin bands of higher stress perpendicular to each other were observed (Fig. 1c). The average von Mises Stress, 95th percentile of (Mises) stress and total strain energy were slightly larger under the shear load setup compared to the compressive load setup (Table 1). All three values were larger than the compressive or shear model in the original load set up (Table 1).

In the compartmentalised model, under the original load setup the stress dissipated throughout the model from the corner where the load was applied to the constrained corner (Fig. 1d). While under the compressive load setup, the area of higher stress was restricted to the top of the model where the load was applied (Fig. 1e) and under the shear load setup, the area of high stress spread from the right hand side near the constrained corner (Fig. 1f). The average von Mises stress, 95th percentile of (Mises) stress and the total strain energy were largest under sole shear loads and smallest in the compressive load model, with values for the original set up falling in between (Table 1).

Note the shear load in the compartmentalised model was applied differently to the arrangement for the biologically realistic model. As the compartmentalised model was not able to run under a sole shear load, like the biologically realistic model, a small constraint on the opposite face (1 µm) was added to help stabilise the model (Fig. 1f).
Here we have demonstrated that the geometric models were more susceptible to shear loads than compressive loads, however the differences were minimal (Table 1). Similar differences between compressive and shear systems have been documented previously in another filamentous cellular material, wood. The fatigue properties of wood laminates were found to be an order of magnitude higher in compressive strength than shear strength (Bonfield and Ansell, 1991). This was also the case with nacre found in abalone shells, an organic-inorganic composite material made of mainly aragonite, which similarly had an order of magnitude difference between shear and compressive strength (Menig et al., 2000).

The compartmentalised model under sole shear or sole compressive loads was less accurate at representing the more biologically realistic model. This was seen in the stress distributions, which were only comparable between the biologically realistic and the compartmentalised models under the original load setup (Fig. 1). The compartmentalised model was especially inaccurate at representing the biologically realistic model under the shear load setup. The computed strain energies were too high for the model to run under a purely shear load and therefore an extra constraint was required to help stabilise the compartmentalised model (Fig. 1f). The regular cells in the compartmentalised model stabilised the model under the compressive load setup, whereas under a shear load setup the individual cells could not help stabilise the model in the direction of the shear load. This highlights the importance of natural variability in the biologically realistic model, which was missing from the regular compartmentalised model making it more susceptible to shear loads.

Interestingly, for the biologically realistic model, the largest results occurred under the original load (a combination of both shear and compressive loads), whereas for the compartmentalised model the largest results occurred under the shear load. This finding highlights the susceptibility the compartmentalised model had to shear loads compared to the biologically realistic model. However it is important to note that the forces used were derived based on experiments on flexible macroalgae found in the wave-swept intertidal zone. Rhodoliths are made up of high Mg-Calcite and are found unattached on the bed. Hence they will experiences different drag forces compared to flexible attached wave swept macroalgae at similar velocities.

The values given in section 2.1 dont add up and the geometry specification is confusing. This is partly due to the Ragazzola paper, but the models need to be clearly specified. Ive attached a figure with best that I could make of the model by examining the figures. Also it needs to be cleared noted that the in the y direction each layer has the same thickness (i.e. the top and bottom surface has the same thickness as the inner ones) but in the x direction the sides (left most and right most walls) have half the thickness of the inner ones. That is, the model is not translations of a unit cells.

The model dimensions are the same as in the Ragazzola paper to enable effective comparison. The measurements used by the reviewer to recreate the model are different from the measurements stated in our paper and hence that is why the reviewer’s dimensions do not add up to our total. We have rechecked our figures and taking into account rounding (as Abaqus can
only measure to 2 decimal places) they do add up. The authors suggest the following changes to the manuscript to avoid any open questions which might lead to this kind of misunderstanding.

Page 6 Line 114
The inter-wall thickness was the thickness of a single cell wall in the x direction (between filaments). Therefore the internal walls of the model had two inter cell walls, whereas the external walls (the left outermost and right outermost) only had one inter cell wall each. The intra-cell thickness was the cell wall thickness between cells in the y-direction. As the rhodolith grows as a set of filaments, there was only one cell wall between two cells in the y-direction (Fig. 2).

Graphing the results makes assessment much easier. I've done a quick plot of the data as an example. Is there any different between 2D and 2D 422 µatm results and the 3D compartment and 3D compartment 422 µatm results? Other than a change in the 5 sig fig for the strain, these two sets of results appear identical and there is no difference between the values and nothing in the text to indicate that these are distinct results. This should be removed from the table.

There is no difference in the results; they are the same, but to make it easier to understand the table we duplicated entries. We understand the reviewer’s comments, however we believe the graph makes the information harder to understand and hence we have kept the table.

After the effort was made to create a 3D model of the actual skeleton, it would be good to have some information about the geometry of the skeleton. It is hard to judge from figure 6, but it would appear that the skeleton has a structure more like Swiss cheese than a regular lattice.

The authors acknowledge the reviewer’s comments about the geometry of the biological model. Please find below our additions to the manuscript.

Page 7 Line 133
This cube was selected at random within the scan of the rhodolith (Fig. 3 a-d), as using the CT scan the summer winter layers could not be distinguished.

Page 10 line 201
Comparison of the internal morphology between the compartmentalised model and the biologically realistic model showed similarities. Both models had regularly distributed cavities. However unlike the compartmentalised model the biological model cavities were spheroidal and, due to the natural variation within these specimens, the arrangement of cavities was not as regimented as in the compartmentalised model (Fig. 4). Both the biologically realistic and the compartmentalised model had the same percentage volumes of calcite and cavities whereas the corridor model had a lower percentage volume of calcite (Table 2).
The importance of these structures is highlighted by the geometric model with compartments (the compartment model) being the most stable of the geometric structures assessed and also most comparable - in terms of percentage volume of calcite, stress distribution and magnitudes of average stress and total strain energy - to the biologically realistic model. This highlights the importance of geometry changes, which our method accurately captured, to the distribution and magnitude of stress.

Should the total strains for each model be compared to the same total stress and the data interpreted as a linear section of a stress-strain curve? Can the results be interpreted in terms of changes in brittleness? How sure are we that the data is on the linear part of the curve? For example, maybe the stresses in the perturbative regime? What would happen if the applied force is varied? I imagine that the geometry dictates the stress strain relationship. Can the models be run with increasing loadings until a fracture occurs, or is this beyond the capability of the software?

The analysis used here employs a linear elastic model, so stress and strain will vary in a linear fashion as dictated by the Young's modulus. Loads can be increased until values that would induce fracture are reached but the overall pattern of stress and strain will remain the same, as will the relative differences between the models with varying geometry. As our aim here is to compare the relative performance of different model types, we have elected not to present a series of further analyses with varying loads. Furthermore, the fracture properties for coralline algae are not well understood and fracture stress and strain is unknown. Further experimental work is required to obtain accurate fracture properties.

Assuming that all the results lie on the linear section a stress-strain curve, can it be concluded that the biological model has a different bulk modulus of elasticity. Even if this holds the modulus of elasticity isn't that relevant to the fracture process, which I would guess is the more relevant quantity. The von Mises is a measure of local stress, and therefore has a distribution across the model. In a fracturing process the extremes of the distribution are important, not the average of the distribution. I think it would be good to include in the table something like the 90th or 95th percentile of the von Mises stresses.

As these models are representing the same organism, with similar volumes, it is assumed that the elastic modulus is the same. Hence this study is effectively analysing the effect of shape on the structural integrity.

The reviewer makes a valid point; indeed in the fracturing process the extremes of the distribution are important. Hence we have added the 95th percentile for the three comparison models (the 3D corridor, 3D compartmentalised and the biologically realistic model to our main table (Table 3).
The 95th percentile of (Mises) stress was additionally used as a comparison between the 3D corridor, compartmentalised and biologically realistic model as this metric highlighted the extremes of the von Mises Stress distribution - an important parameter to highlight fracture potential.

This was also seen with the 95th percentile of (Mises) stress which showed the compartmentalised and the biologically realistic model result in similar values. Whereas the corridor model resulted in both the 95th percentile of (Mises) stress and the strain energy an order of magnitude higher, while the average stress was over double the amount than both the compartmentalised and biologically realistic model (Table 3).

Also, rather than plotting the stresses over the entire 3D model (which shows only the surface stresses) could the areas experiencing the highest stresses be identified? For example, the compartment might have a maximum on in the middle of the left facing surface while the biological model might have a maximum in the middle of the right facing surface. Similarly, the most informative part of the 3D stress might be a slice through the centre. The plots in paper show the stresses on the surface which are affect by the conditions - I would expect these surface layer stress to most sensitive to simulation details like size of model, while the central ones would be more stable. On the other hand, a fracture probably starts at the surface and propagates inwards, in which case the surface stresses are most relevant.

As the surface layers are directly in contact with the loads and the constraints this is where the model is most sensitive, hence why the minimum and maximum stresses were located on the surface (Fig. 5). The minimum and maximum values, therefore, do not provide much more information than already provided by the surface plots and hence are not discussed further. We checked, and the stress distribution does not change throughout the model hence we have chosen not to figure this further in the revised manuscript.

Stress results were also displayed as scaled colour plots. Stress distribution throughout the model was very similar to surface stress distribution. The surfaces of the model were more sensitive to the loads and constraints, due to immediate contact with the boundary conditions. The minimum and maximum von Mises stress values were found on the surfaces of the models, being more influenced by the position of the boundary conditions and complexities in the geometry. Hence, the minimum and maximum values did not provide any additional information on the overall structural integrity of the model than that provided by the surface contour plots.
What is the relationship between in the von Mises stress and the total stress? Do the von Mises stress integrate up to the total stress? Would this total stress be the force over the area of the skeleton in contact with the boundary, or would it be bulk area?

Von Mises stress, a function of each of the principle stresses which represent compressive or tensile stress, is a parameter used to determine failure i.e. if the maximum value of von Mises stress induced in the material is greater than the failure stress of the material the design will fail. However we are not using the parameter to determine failure but to compare relative stress between different structures. So here von Mises stress is the total stress and the total stress is the force over the bulk area.

If the model is converged with respect to mesh points the results wont depend on the number of elements - that is, it doesn't matter whether a triangular, hexagonal, random etc. mesh is used the answer should be same. Comparing the results for 3D corridor with a hexagonal mesh (1 × 10^6 elements) with the tetrahedral mesh (2 × 10^6) the total strain differs by an order of magnitude. This indicates that one (or more likely both) of these models is far from convergence. What checks of convergence has been done? Without being sure that the results are converged how is it possible to compare the results of one geometry to another?

A convergence test was performed on both meshes, where the mesh size was subsequently decreased until the average von Mises stress became constant. We now include these analyses in the paper. Theoretically the different type of elements, once converged, should not have an impact on the distribution of the stress. However in reality the stiffness of the tetrahedral mesh, with better interlocking elements, does affect the results. Please see below for our addition to the manuscript.

Page 7 line 145
Convergence test were performed for each mesh type in order to determine the minimum mesh size required. The mesh size was decreased until the average von Mises value no longer changed relative to mesh size. Hypothetically, all refined meshes should converge to similar results yet our converged von Mises stress value was an order of magnitude different between the hexagonal and tetrahedral mesh. This was due to the shape of the tetrahedral elements and the way tetrahedral elements interlock together, making a tetrahedral model stiffer than a hexagonal model. This is reiterated by Dumont et al. (2005), who found that comparing a converged 4-node linear and a stiffer 10-node quadrilateral tetrahedral mesh of the same model gave different mean stress values, but within 10%. In order to compare the 2D to 3D geometric models, the corridor model was meshed with 4-node linear hexagonal elements. As tetrahedral elements were better at capturing the complex geometry of the biological model, all models were then meshed with 4-node linear tetrahedral elements in order to be compared to the biologically realistic model and to each other.
Related to the last point, no uncertainties given. A simple assessment of the variation of the results with change in cell size parameters or number of grid points a rough indication of variations.

In response to the first point, we now provide results that indicate how sensitive the model is to changes in material properties.

Varying cell size parameters represents a substantial amount of work and is beyond the scope of this paper but is certainly something to follow up in future work. However care was taken in choosing the cell sizes used in the study. A significant number of cells were measured under SEM in both current and year 2050 conditions and the average measurements were used to create the model geometries here.

It is not clear what the reviewer means by number of grid points and therefore we are not able to respond.

Page 8 line 154
Initially, to analyse how sensitive the models were to changing material properties, a set of 2D and 3D corridor models with different Young’s modulus (maximum and minimum Young’s modulus values of two different bivalves - *Mytilus edulis* and *M. californianus*) were analysed.

Page 11 line 208
The sensitivity test of the 2D model and the 3D corridor model highlighted that increasing the Young’s modulus by 120% did not result in any change in stress, whereas the total strain energy decreased with increasing Young’s modulus (Table 4).

Page 14 line 280
However it is important to note that the sensitivity test highlighted that the Young’s modulus only had an effect on the total strain energy (Table 4) as stress is directly proportional to force and inversely proportional to area while strain is inversely proportional to the Young’s Modulus (equation 2). Hence increasing the Young’s modulus caused a decrease in the total strain energy - as seen in other studies on organisms such as macaques (Strait et al., 2005), alligators (Reed et al., 2011) and pig skulls (Bright and Rayfield, 2011). Although structures with heterogeneous material (in this case seasonality affecting Mg concentrations) did display differences in both stress and strain (Reed et al., 2011; Strait et al., 2005).
References
Table 1: Average von Mises Stress, 95\textsuperscript{th} percentile of (Mises) stress and total strain energy for the different load types exerted on the biologically realistic, compartmentalised and corridor model. Total strain energy for the biologically realistic model has been corrected for calcite volume (equation 1). The compartmentalised model under the shear loading type is highlighted in grey to reiterate that the loading setup is different to the biologically realistic model under a shear loading type.

<table>
<thead>
<tr>
<th>Model</th>
<th>Loading Type</th>
<th>Average von Mises Stress (N ( \mu \text{m}^{-2} ))</th>
<th>95\textsuperscript{th} Percentile of (Mises) Stress (N ( \mu \text{m}^{-2} ))</th>
<th>Total Strain Energy (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biologically realistic</td>
<td>Compressive</td>
<td>2.34E-04</td>
<td>4.08E-04</td>
<td>2.99E+05</td>
</tr>
<tr>
<td></td>
<td>Shear</td>
<td>2.83E-04</td>
<td>6.07E-04</td>
<td>5.47E+05</td>
</tr>
<tr>
<td></td>
<td>Original (Shear + Compressive)</td>
<td>3.49E-04</td>
<td>7.21E-04</td>
<td>8.71E+05</td>
</tr>
<tr>
<td>Compartmentalised</td>
<td>Compressive</td>
<td>2.42E-04</td>
<td>2.96E-04</td>
<td>2.34E+05</td>
</tr>
<tr>
<td></td>
<td>Shear</td>
<td>4.27E-04</td>
<td>1.03E-03</td>
<td>1.63E+06</td>
</tr>
<tr>
<td></td>
<td>Original (Shear + Compressive)</td>
<td>4.10E-04</td>
<td>8.25E-04</td>
<td>9.63E+05</td>
</tr>
</tbody>
</table>

\* Strain energy corrected for calcite volume
Table 2: Percentage volumes of calcite and cavities in the biologically realistic model, the corridor and the compartmentalised model.

<table>
<thead>
<tr>
<th>Material</th>
<th>Biologically Realistic Model</th>
<th>3D Corridor</th>
<th>3D Compartmentalised</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume of Calcite (µm³)</td>
<td>3.09E+05</td>
<td>1.94E+05</td>
<td>2.87E+05</td>
</tr>
<tr>
<td>Volume of Cavities (µm³)</td>
<td>1.83E+05</td>
<td>2.60E+05</td>
<td>1.67E+05</td>
</tr>
<tr>
<td>Percentage of Calcite</td>
<td>63%</td>
<td>43%</td>
<td>63%</td>
</tr>
<tr>
<td>Percentage of Cavities</td>
<td>37%</td>
<td>57%</td>
<td>37%</td>
</tr>
</tbody>
</table>

Table 3: Mesh type, number of elements, average von Mises stress, 95<sup>th</sup> percentile of (Mises) Stress and total strain energy for the different models.

<table>
<thead>
<tr>
<th>Model</th>
<th>Mesh Type</th>
<th>Number of Elements</th>
<th>Average von Mises Stress (Pa)</th>
<th>95&lt;sup&gt;th&lt;/sup&gt; Percentile of (Mises) Stress (Pa)</th>
<th>Total Strain Energy (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>422 µatm 2D model</td>
<td>Quad</td>
<td>16368</td>
<td>1.67E+11</td>
<td></td>
<td>8.91E+08</td>
</tr>
<tr>
<td>589 µatm 2D model</td>
<td>Quad</td>
<td>1889</td>
<td>5.17E+11</td>
<td></td>
<td>1.27E+10</td>
</tr>
<tr>
<td>3D Corridor</td>
<td>hexagonal</td>
<td>202304</td>
<td>1.72E+11</td>
<td></td>
<td>1.11E+11</td>
</tr>
<tr>
<td>3D Corridor *</td>
<td>hexagonal</td>
<td>202304</td>
<td></td>
<td></td>
<td>4.79E+11</td>
</tr>
<tr>
<td>3D Corridor (model 1)</td>
<td>tetrahedral</td>
<td>2125549</td>
<td>7.46E+10</td>
<td>2.21E+11</td>
<td>1.75E+10</td>
</tr>
<tr>
<td>422 µatm Compartmentalised (model 2)</td>
<td>tetrahedral</td>
<td>3442433</td>
<td>2.75E+10</td>
<td>5.36E+10</td>
<td>4.28E+09</td>
</tr>
<tr>
<td>Biologically realistic model (model 3)</td>
<td>tetrahedral</td>
<td>2106858</td>
<td>2.74E+10</td>
<td>5.63E+10</td>
<td>5.21E+09</td>
</tr>
<tr>
<td>589 µatm Compartmentalised (model 4)</td>
<td>tetrahedral</td>
<td>1707673</td>
<td>4.98E+10</td>
<td></td>
<td>8.81E+09</td>
</tr>
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</table>
Table 4: Average von Mises Stress and total strain energy for the comparison of the different material properties in the 2D and 3D corridor models.

<table>
<thead>
<tr>
<th>Material</th>
<th>Young’s Modulus (Pa)</th>
<th>Average von Mises Stress (Pa)</th>
<th>Total Strain Energy (J)</th>
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</thead>
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<tr>
<td><strong>2D</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Calcite</td>
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<td>1.67E+11</td>
<td>8.91E+08</td>
</tr>
<tr>
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<tr>
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<td>1.67E+11</td>
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<tr>
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<td>4.04E+08</td>
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<td>M. californianus -max</td>
<td>7.93E+10</td>
<td>7.46E+10</td>
<td>7.94E+09</td>
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</table>
Figure 1: The von Mises Stress patterns on the biologically realistic model (a, b & c) and the compartmentalised model (d, e & f) in different loading situations; the original (mixture of both shear and compressive loads) (a & d); compressive load (b & e) and the shear load (c & f). The different load situations (pink box) are shown on the biologically realistic model as well as the constraints (green boxes). The shear load for the compartmentalised model was set up slightly differently and hence the loading setup is displayed on the model. Units = N $\mu$m$^{-2}$
Figure 2: Dimensions used in the 3D corridor model. Units = μm
Figure 3: Orthoslice projections in the different planes of a rhodolith thallus: a) xy direction; b) xz direction; c) yz direction and d) in 3D format. The purple box highlights where the 80µm x 80µm cube was selected.
Figure 4: The inside spheroidal cavities of the biologically realistic cube
Figure 5: The minimum and maximum von Mises stress values of a) the corridor model, b) the compartmentalised model and c) biologically realistic model. Units = Pa.
Potential and Limitations of Finite Element Modelling in Assessing Structural Integrity of Coralline Algae under Future Global Change

Running head: Modelling the coralline algal skeleton

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ABSTRACT

Coralline algae are important habitat formers found on all rocky shores. While the impact of future ocean acidification on the physiological performance of the species has been well studied, little research has focussed on potential changes in structural integrity in response to climate change. A previous study using 2D Finite Element Analysis (FEA), suggested increased vulnerability to fracture (by wave action or boring) in algae grown under high CO₂ conditions. To assess how realistically 2D simplified models represent structural performance, a series of increasingly biologically accurate 3D FE-models that represent coralline algal growth were developed. Simplified geometric 3D models of the genus Lithothamnion were compared to models created from computed tomography (CT) scan data of the same genus. The biologically accurate model and the simplified geometric model representing individual cells had similar average stresses and stress distributions, emphasizing the importance of the cell walls in dissipating the stress throughout the structure. In contrast models without the accurate representation of the cell geometry resulted in larger stress and strain results. Our more complex 3D model reiterated the potential of climate change to diminish the structural integrity of the organism. This suggests that under future environmental conditions the weakening of the coralline algal skeleton along with increased external pressures (wave and bioerosion) may negatively influence the ability for coralline algae to maintain a habitat able to sustain high levels of biodiversity.
1 INTRODUCTION

Since the pre-industrial era CO$_2$ concentrations have risen by more than 100ppm (Ciais et al., 2013). The ocean has absorbed approximately 30% of the total anthropogenic CO$_2$ emissions (Rhein et al., 2013). This has led to a change in the carbonate chemistry of the ocean, resulting in a lowering of the carbonate saturation state and a decrease in ocean pH, a process termed ‘ocean acidification’ (Caldeira and Wickett, 2003). Future projections suggest an additional average decrease in pH of between 0.13 (RCP 2.6) and 0.42 units (RCP 8.5) by the year 2100 (Pörtner et al., 2014). Calcifying organisms are at risk as the lower carbonate saturation state may affect the ability to calcify shells and skeletons (Diaz-Pulido et al., 2011; Fabricius et al., 2011; Kroeker et al., 2010). In response, ecosystem shifts are predicted to occur; for example sea-grasses and kelps may outcompete calcifying algae as the major habitat formers in shallow water habitats (Fabricius et al., 2011).

Rhodoliths (Fig. 1), non-geniculate free living (live and dead) coralline red algae (Foster, 2001), are an extremely diverse group of benthic calcifying organisms found from the polar to the tropical regions and low intertidal zones to 150m deep (Foster, 2001). They are major contributors to the global inorganic carbon budget in shallow water ecosystems (Mackenzie et al., 2004). The largest rhodolith bed found on the Abrolhos shelf contributes roughly 5% to the global calcium carbonate budget (Amado-Filho et al., 2012). Additionally these benthic ecosystems support a high level of biodiversity by forming structurally and functionally complex habitats (Nelson, 2009) for many organisms including polychaetes, crustaceans and molluscs (Foster, 2001), as well as being important nursery grounds to commercial species including scallops (Grall and Hall-Spencer, 2003). Coralline algae biodiversity and habitat complexity are directly correlated; species richness and abundance of, in particular, arthropods, annelids and cnidarians, are known to increase as rhodolith size and branching increases (Foster et al., 2013). However coralline algae are ecologically fragile due to their
slow growth rate of ~1mm yr\(^{-1}\) (Freiwald and Henrich, 1994) and sensitivity to physical disturbances such as extraction and dredging.

Furthermore, as they form high Mg- calcite skeletons, the most soluble polymorph of calcium carbonate (CaCO\(_3\)) (Andersson et al., 2008), coralline algae are more susceptible to ocean acidification than other organisms that utilise different polymorphs of calcium carbonate. Consequently, a significant loss of rhodoliths from the North Atlantic by the year 2100 has been predicted (Brodie et al., 2014). However, laboratory experiments have shown highly varied responses to elevated levels of CO\(_2\). Coralline algae (Neogoniolithon sp.) and calcareous green algae (Halimeda incrassata) exposed to increased CO\(_2\) concentrations (606 and 903 ppm for 60 days) showed an increase in calcification (Ries et al., 2009); whereas another study on Lithothamnion glaciale, a cold temperate coralline algae, displayed a decrease in growth rate at elevated CO\(_2\) conditions (589 and 755 µatm for three months) (Ragazzola et al., 2012), while also showing evidence for potential acclimation after ten months (Ragazzola et al., 2013).

Coralline algae have many pressures to withstand in their natural environments, including wave action and bioerosion, while maintaining a structurally and functionally complex habitat. Under elevated CO\(_2\) conditions the algal skeleton appeared to be weakened (Ragazzola et al., 2012), thereby favouring erosion and breakage (Kamenos et al., 2013; Ragazzola et al., 2012).

Finite Element Analysis (FEA) is a technique that reconstructs the stress, strain and deformation in structures (Zienkiewicz et al., 2005). Originally developed for mathematical and engineering applications, it has recently become an established technique in zoology and palaeontology to understand morphology, function and evolution of hard tissue structures (Rayfield, 2007).
FEA works by transforming a continuous structure into a discrete number of elements which are connected to each other via nodes. The combination of elements and the interconnecting nodes form the mesh. Appropriate material properties (Young’s modulus and Poisson’s ratio) are assigned to the elements to mimic the elasticity of the structure. Adequate boundary conditions (magnitude and direction of loading and constraints) are applied and then nodal displacements are calculated in response to the applied boundary conditions and material properties of the model. The nodal displacement is used to calculate the strain and subsequently stress (using the Young’s modulus, see equation 1) and hence mechanical performance of complex structures can be inferred. (For mathematical equations see Mathematics of FEA, (Rayfield, 2007) supplementary material)

\[
E \text{ (Young’s modulus)} = \frac{\sigma \text{ (stress)}}{\varepsilon \text{ (Strain)}}
\]

(1)

Ragazzola et al. (2012) were the first to use FEA in ocean acidification studies by creating simple 2D FE-models of coralline algae to quantify the effect of elevated CO_2 on the skeleton of *Lithothamnion glaciale*. Although the changes in growth rate were not significant, specimens grown under CO_2 conditions predicted for the year 2050 were found to have significantly larger cells and thinner cell walls. These ultrastructure changes resulted in predicted increased vulnerability to fracture compared to present day structures (Fig. 2a-b) as observed in the 2D FE-model.

These simple 2D models represented the mechanical performance of a cross-section of uniform thickness (1µm) (Ragazzola et al., 2012) through the algal structure. However, these highly innovative models were simplistic in nature. Importantly, they had not been tested to assess if they were a fair representation of skeletal mechanical performance. Consequently, the simple 2D model may have overestimated the distribution and magnitude of stress and hence future vulnerability of algal communities. Here we have developed a set of 3D FE
geo
metric models to represent different aspects of coralline algae morphology and compared
these models with a more biologically accurate 3D FE-model generated from computed
tomography (CT) data, allowing us to assess the trade-off between computing time (Andersen
and Jones, 2006; Romeed et al., 2006) and the need for an appropriate representation of the
structure.

We then used our improved understanding of the performance of the 3D models to re-assess
the impact of current and future ppm CO₂ induced changes to morphology on the ability of
algal communities in maintaining a high level of biodiversity. Finally, the loading and
constraint taken from Ragazzola et al. (2012) were a mixture of shear and compressive
forces, which simulated boring forces by an organism exerted on the exposed corner of an
attached thallus. As it was assumed that these organisms were more prone to shear forces
than compressive ones, we also assessed the effect of sole compressive or sole shear forces
on the compartmentalised and the biologically realistic model.
2 MATERIALS AND METHODS

2.1 Model Geometry

Four 3D FE-models with different coralline algal features were created based on the measurements and properties of Ragazzola et al. (2012) models. All 3D geometric models were created and analysed in the Finite Element software package, Abaqus/CAE, v.6.10, (Simula, USA, Dassault Systémes, //Simula, Providence, RI, USA), following the protocol established by Ragazzola et al (2012).

Firstly, the 2D models from Ragazzola et al. (2012) were expanded, using the extrude command in Abaqus, to create a 3D model with the same area, all length and width dimensions (78.92 x 72.46 µm) were unchanged, but a depth of 80 µm was used instead of 1 µm. Size of the individual cells mirrored that of the 422 µatm model of Ragazzola et al. (2012), with an individual cell height of 6.3 µm and width of 5.15 µm and an inter- and intra-wall thicknesses of 2.68 µm and 0.86 µm respectively. This structure is assigned the label ‘Corridor model’ (Fig. 3a). The inter-wall thickness was the thickness of a single cell wall in the x direction (between filaments). Therefore the internal walls of the model had two inter-cell walls, whereas the external walls (the left outermost and right outermost) only had one inter cell wall each. The intra-cell thickness was the cell wall thickness between cells in the y-direction. As the rhodolith grows as a set of filaments, there was only one cell wall between two cells in the y-direction (Fig. 4).

2.1.1 Compartmentalised (Individual Cell) model with current/422 µatm CO₂ cell size

Although the cellular width and height in the Corridor model represented the dimensions recorded by Ragazzola et al. (2012) from SEM images, the cells are not represented as discrete entities and instead are represented as hollow calcite ‘tubes’. As algal cells grow apically, they form a lattice of individual cells, hence a second model, ‘Compartment model’ (Fig. 3b) was created in which cell walls were added in the Z direction to create discrete
cellular spaces. All dimensions apart from the depth of the individual cells and divisions
between cells in the z direction were the same as the corridor model. It was assumed the
depth of the cell was the same as the width (5.15 μm) and the divisions between the cells in
the z direction were the same as the inter cell wall divisions (2.68 μm).

2.1.2 Biologically realistic model
Computed tomography (CT) scans of L. glaciale derived from the TOMCAT beamline at the
Swiss Light source, Switzerland (see Ragazzola et al, 2012) were imported into Avizo V8.0
(VSG) software package. Each scan was 150 projections over 180° using a 15 keV energy
beam with UPLAPO 10x objective. The field of view was 1.5 x 1.5mm² and the pixel size
was 0.74 x 0.74 mm². A 3D cube of similar dimensions (80 x 80 x 80 μm) as the geometric
models was created but instead capturing the actual morphology of the rhodolith (known as
the ‘Biological model’) (Fig. 3c). The cube was selected at random within the scan of the
rhodolith (Fig. 5 a-d), as the summer winter layers could not be distinguished in the CT scan.
The scans were thresholded in Avizo and then exported to Hypermesh (v.11; part of the
Hyperworks package from Altair, MI, USA) to create FE-models, which were analysed in
Abaqus v6.10.

2.1.3 Compartmentalised (Individual Cell) model with 2050 / 589 μatm CO₂ cellular
dimension
A final model, ‘the OA model’ (Fig. 3d), was created by adjusting the cell size and spacing to
represent the dimensions of the future 589 μatm CO₂ model as a 3D and compartmentalised
structure with overall dimensions (74.99 x 77.2 x 80 μm), individual cell dimensions (11.7 x
7.99 x 7.99 μm) and intra and inter-cell wall dimensions (0.685 μm and 0.829 μm
respectively). This future predicted CO₂ model was compared to the performance of the 3D
compartment model with current CO₂ ppm cell size.
2.2 Finite element analysis (FEA)

2.2.1 Boundary conditions

In keeping with Ragazzola et al. (2012), a load pressure of 20,000 Pa was applied to the top left corner, 40 µm along the external top surface and 40 µm down the left surface of the models. Constraints were applied to the whole bottom surface and on the right hand surface, opposite the loads, 40 µm up from the constrained bottom (Fig. 6). This simulated the attachment of the structure to the rest of the thallus. Even though the same loads as Ragazzola et al. (2012) were used, it is known that the primary hydrodynamic force exerted on marine macroalgae is drag force (Carrington, 1990). Drag ($F_{\text{drag}}$) force can be calculated using equation 2.

$$F_{\text{drag}} = \frac{1}{2} (\rho U^2 A C_d)$$

Where $\rho$ is the seawater density (approximately 1025 kg m$^{-3}$); and $U$ is water velocity. Subtidal marine macroalgae experience a water velocity on the order of magnitude of 1 m s$^{-1}$ (Carrington, 1990), while intertidal species can experience breaking waves of up to 25 m s$^{-1}$ (Denny et al., 2003). $A$ is the algal planform area; and $C_d$ the drag coefficient (dimensionless index of shape change and reconfiguration of flexible fronds (Carrington, 1990; Dudgeon and Johnson, 1992; Gaylord et al., 1994)). However, there are no data published for resulting in breakage of rhodoliths, instead the existing literature focused on flexible macroalgae, making it difficult to find loads that are environmentally significant whilst also being species related.

2.2.2 Element type and size

Convergence tests were performed for each mesh type in order to determine the minimum mesh size required. The mesh size was decreased until the average von Mises value no longer changed relative to mesh size. Hypothetically, all refined meshes should converge to similar results yet our converged von Mises stress value was an order of magnitude different between the hexagonal and tetrahedral mesh. This was due to the shape of the tetrahedral elements and
the way tetrahedral elements interlock together, making a tetrahedral model stiffer than a hexagonal model. This is reiterated by Dumont et al. (2005), who found that comparing a converged 4-node linear and a stiffer 10-node quadrilateral tetrahedral mesh of the same model gave different mean stress values, but within 10%. In order to compare the 2D to 3D geometric models, the corridor model was meshed with 4- node linear hexagonal elements. As tetrahedral elements were better at capturing the complex geometry of the biological model, all models were then meshed with 4-node linear tetrahedral elements in order to be compared to the biologically realistic model and to each other.

### 2.2.3 Material Properties

In keeping with Ragazzola et al. (2012), all models were assumed to be composed of a linearly elastic, isotropic, homogeneous material with a Young’s Modulus of 36 GPa and a Poisson ratio of 0.31 (properties of calcite) (Tanur et al., 2010). Even though these material properties do not accurately represent the heterogeneities in the specimen and their material properties this approach gives a comparative insight into how different geometries affect the overall strength of a structure.

Initially, to analyse how sensitive the models were to changing material properties, a set of 2D and 3D corridor models with different Young’s modulus (maximum and minimum Young’s modulus values of two different bivalves – *Mytilus edulis* and *M. californianus*) were analysed.

### 2.2.4 Displaying and recording results

In order to compare the impact of predators on the different 3D geometric models, stress and strain results were calculated. Stress, generated by the applied load (force) on a given area, is represented by the von Mises stress, a function of each of the principle stresses that represents tensile or compressive stress (Rayfield, 2007). Average stresses were calculated by dividing the von Mises stress by the element size to account for differing number of elements between...
models. Total strain energy refers to the energy stored in a system as a load is applied, which is a useful variable to record in mineralized structures as the more strain in a system leads to a larger amount of potential energy available for fracture (Gordon, 1978). The 95th percentile of (Mises) stress was additionally used as a comparison between the corridor, compartment and biological models as this metric highlighted the extremes of the von Mises Stress distribution – an important parameter to highlight fracture potential.

Stress and strain energy are linearly dependent on surface area and volume respectively (Dumont et al., 2009), hence the applied pressure was rescaled for the biological model to rule out the effect of increased Mg-calcite volume on modelling stress and strain. To account for the increase in volume of calcite between the 2D and the 3D model, as strain energy is dependent on volume, the strain energy was calculated for the 3D model using equation 3, outlined by Dumont et al. (2009);

\[ U_B^* = \frac{V_B}{V_A}^{1/3} \left( \frac{F_A}{F_B} \right)^2 U_B \]  

where \( U_B^* \) is the total strain energy for model B corrected for calcite volume, \( V_A \) and \( V_B \) are the volumes for the models A and B respectively, \( F_A \) and \( F_B \) are the loads applied to the two models and \( U_B \) is the total strain energy for model B.

The von Mises stress was displayed graphically on the model, with warm colours (red/orange) indicating areas of high stress and cool colours (blue) indicating areas of low stress. Stress distribution throughout the model was very similar to surface stress distribution. The surfaces of the model were more sensitive to the loads and constraints, due to immediate contact with the boundary conditions. The minimum and maximum von Mises stress values were found on the surfaces of the models, being more influenced by the position of the boundary conditions and complexities in the geometry. Hence, the minimum and maximum values did not provide any additional information on the overall structural integrity of the
model than that provided by the surface contour plots. Average stress values, total strain energies and 95th percentile of (Mises) stress can be found in Table 1.

2.3 Comparison between shear and compressive loading

The biological and the compartment model were exposed to different loading scenarios in Abaqus. This included the original load setup explained earlier in section 2.1.4 (Fig. 7a); the compressive loads, where the load was applied to the top of the cube opposite the constraint (Fig. 7b); and shear loads, where the load was applied on the face adjacent to the bottom constraint (Fig. 7c).

As this part of the study moved on from the initial research of Ragazzola et al. (2012), it was decided to use loads defined experimentally based on real wave velocities. Starko et al. (2015) used wave velocities of up to 3.5 m s\(^{-1}\) to assess the effect of branching in flexible wave swept macroalgae, in which they also measured the drag force. Water velocity experienced by subtidal marine macroalgae is on the order of magnitude of 1 m s\(^{-1}\) (Carrington, 1990). Hence, we used a drag force (0.9N) corresponding to a wave velocity of 3.5 m s\(^{-1}\) experienced by a heavily branched macroalgae (similar in branching to our rhodoliths) to carry out our load type comparison tests. Here we have kept the force per unit area constant in order to compare the compartment model to the biological model. Strain energy is dependent on volume, therefore in order to compare the total strain energy between the models we had to take into account the difference in calcite volume between the biological model and the compartment model (using equation 3) (Dumont et al., 2009).
3 RESULTS

A similar stress distribution was observed in the 2D and 3D geometric model (Corridor model) (Fig. 8a-b), with areas of high stress occurring along the intra-cell walls and adjacent to the constrained surface. The average von Mises stress of the two models was very similar (Table 1), indicating that the simple 2D model (Fig. 8a) was an accurate representation of the stress in a simple 3D geometric model (Fig. 8b). As expected, the total strain energy in the 3D model was over 500x higher than the 2D model, showing that the 3D model with a larger volume was able to store more than the relative amount of strain energy compared to the 2D model.

Once the complexity of the 3D model was increased to better represent the natural structural complexity, differences between the simple 3D model (Corridor model) and the more complex models became evident (Fig. 9a-c). In the compartment model of current CO₂ conditions (Fig. 9b), both, the average stress and total strain energies were lower than the Corridor model (63% and 76% respectively) (Table 1). The addition of cellular walls in the Z direction removed regions of peak stress near the constraints as stresses now dissipated throughout the structure, stabilising the model.

The results of the comparison between the 3D geometric models (the Corridor and Compartment model) and the realistic model of similar dimensions generated from CT scans (the Biological model) showed that a similar average stress, strain energy (Table 1) and stress distribution (Fig. 9b-c) was observed between the compartment model and the biological model. Comparison of the internal morphology between the compartment model and the biological model also showed similarities. Both models had regularly distributed cavities. However unlike the compartment model the biological model cavities were spheroidal and, due to the natural variation within these specimens, the arrangement of cavities was not as regimented as in the compartment model (Fig. 10). Both the biological model and the
compartment model had the same percentage volumes of calcite and cavities whereas the corridor model had a lower percentage volume of calcite (Table 2).

As the compartment model was similar in performance to the biological model, we used this model to assess the impact of ocean acidification (Fig. 2c-d). The change in wall thickness and cell size in the 589 µatm/2050 model led to a near doubling of average stress and a doubling of the total strain energy (Table 1). This increase in strain energy was less than suggested using the simple 2D models, which predicted an increase in average stress of 309% and strain energy of 1421%.

However, using more environmentally significant forces in the shear and compressive comparison tests, we can see that the stresses and strains exerted by these organisms were not as large as those taken from Ragazzola et al. (2012). Accounting for the change in units, the differences between the von Mises stress results (Pa) are on the order of magnitude of $10^9$ instead of $10^{11}$.

In the biological model, under the original load setup, stress dissipated throughout the model from the corner where the load was applied to the constrained corner (Fig. 7a). While under the compressive load setup, the stress had a top to bottom distribution (from the loaded surface to the constrained surface) with a slight increase in stress surrounding the cavities in the model (Fig. 7b) and under the shear load setup, two thin bands of higher stress perpendicular to each other were observed (Fig. 7c). The average von Mises Stress, 95th percentile of (Mises) stress and total strain energy were slightly larger under the shear load setup compared to the compressive load setup (Table 3). All three values were larger than the compressive or shear model in the original load set up (Table 3).

In the compartment model, under the original load setup the stress dissipated throughout the model from the corner where the load was applied to the constrained corner (Fig. 7d). While
under the compressive load setup, the area of higher stress was restricted to the top of the
model where the load was applied (Fig. 7e) and under the shear load setup, the area of high
stress spread from the right hand side near the constrained corner (Fig. 7f). The average von
Mises stress, 95th percentile of (Mises) stress and the total strain energy were largest under
sole shear loads and smallest in the compressive load model, with values for the original set
up falling in between (Table 3).

Note the shear load in the compartment model was applied differently to the arrangement for
the biological model. As the compartment model was not able to run under a sole shear load,
like the biological model, a small constraint on the opposite face (1µm) was added to help
stabilise the model (Fig. 7f).

The sensitivity test of the 2D model and the 3D corridor model highlighted that increasing the
Young’s modulus by 120% did not result in any change in stress, whereas the total strain
energy decreased with increasing Young’s modulus (Table 4).
Ragazzola et al. (2012) used 2D FE-models to predict if changes to coralline algae cellular morphology induced by higher CO$_2$ concentrations generate a structure that might less able withstand stresses compared to current specimens. They predicted that increased CO$_2$ and resulting ocean acidification will create a structure more susceptible to fracture in response to environmental stressors such as wave action and boring. This 2D model was the first step in assessing the structural and mechanical consequences of high CO$_2$-induced changes to growth structure, but it is important to note that it represented a 2D approximation of a complex 3D structure. Here our results showed that a 3D extrapolation of the 2D FE-model (Corridor model) generated comparable patterns and magnitudes of average stress to the 2D model. However, once the complexity of the 3D model was altered to approximate cellular compartments and compared to a biologically realistic model generated from SRXTM data, it became clear that stress and strain energy magnitudes in the simple 2D and 3D models were an over-estimation.

Coralline algae grow apically with lateral cell fusion being very common (Irvine and Chamberlain, 1994). This creates a vast network of individual cells able to oppose stress in all directions (Gordon, 1978). The importance of these structures is highlighted by the geometric model with compartments (the Compartment model) being the most stable of the geometric structures assessed and also most comparable – in terms of percentage volume of calcite, stress distribution and magnitudes of average stress and total strain energy – to the biological model (Table 1 & 2). This highlights the importance of geometry changes, which our method accurately captured, to the distribution and magnitude of stress. This occurrence was also observed by Romeed et al. (2006) who previously found that changes in the geometry between their 2D and 3D models of a restored premolar tooth also affected their displacement and profile stresses.
Creating the FE-model of the biologically realistic structure (the Biological model) from SRXTM data was a time consuming process compared to the user effort required to generate the compartmentalised geometric model (the Compartment model). As these different models produced very similar measures of average stress (2.75E+10 Pa compared to 2.74E+10 Pa for the geometric and the biological model respectively), comparable measures of total strain energy (4.28E09 J compared to 5.21E09 J) and a similar stress distribution, we propose that the compartment model presented a time efficient opportunity to manipulate geometric features to test hypotheses of mechanical performance and structural integrity.

Hence this model was used to assess the impact of ocean acidification changes to the cell growth on structural integrity. When the cellular size of the 3D model was adjusted to reflect 589 μatm growth conditions, the structure was less ‘efficient’ than the structure under current environmental conditions, with a higher average stress and total strain energy. It is important to note that the increase in stress and total strain energy magnitude was less pronounced than that suggested by the 2D models, indicating that the extra cellular walls aided in stabilising the thinner cell walls of the model. This finding was in agreement with other work showing that microstructural features affect the mechanical properties of cellular solids (Gibson, 2005). For example, wood has a stiffness to weight ratio equal to steel, which is partly due to the arrangement of cells in a honeycomb structure (Gibson et al., 2010). Hence these features provide habitat forming organisms with a larger structural integrity to withstand external pressures and the ability to live in a highly dynamic environment such as the coastal shelf.

Although our geometric and biological models show congruence, they are still simplifications of the heterogeneities in the algal skeleton. A factor which was not considered in this study is the potential effect of changing material properties due to global change on the algal skeleton. Material properties are affected by the concentration of Mg in the skeleton (Ma et al., 2008), which is dependent on temperature (Kamenos et al., 2008) and potentially pH (Ragazzola et
Mg incorporated into the calcite lattice increases the lattice distortion, which causes an increase in the sliding resistance and deformation resistance to crystals (Wang et al., 1997). Ma et al. (2008) found that due to a much larger difference in mol% of MgCO$_3$ (just below 40%), the high Mg-calcite polycrystalline matrix of the tooth of a sea urchin had a significantly higher elastic modulus ($E$) and hardness ($H$) value than both synthetic calcite and the single crystalline needles.

Sea surface temperatures in the North Atlantic are predicted to rise 2.5°C by the end of the century (Rhein et al., 2013). This rise in temperature corresponds to a 3% increase in mol% of MgCO$_3$ (Kamenos et al., 2008). As a 3% change in mol% of MgCO$_3$ is within the natural variation between specimens (Ragazzola et al., 2013), we suggest such a mol% change in MgCO$_3$ will not significantly impact the material properties and hence affect the skeleton. However, Ma et al. (2008) also suggested the large difference in material properties wasn’t due to just the difference in mol% of MgCO$_3$ but also due to the orientation and uniform size of the crystals. This illustrates that Mg/Ca ratios are not the only factor which affects material properties.

Proteins are also known to affect material properties as the incorporation of organic macromolecules reduces the brittleness and enables plastic deformation (Berman et al., 1988; Ma et al., 2008; Wang et al., 1997; Weiner et al., 2000). The presence of chitin and collagen within the skeleton of Clathromorphum compactum aids calcification and increase skeletal strength (Rahman and Halfar, 2014). Implementing accurate material properties specific for specimens grown under a wide range of conditions into the model would allow a more accurate assessment of whether phenotypic variability in material properties has the potential to counteract structural changes in response to ocean acidification.
However, using these more biologically accurate models, we have further supported previous results that state future climate change will lead to a loss in the structural integrity of coralline algae. We have shown that by increasing the complexity of a simple 2D geometric model to a 3D geometric model we can obtain informative data on the effect of ocean acidification on the structural integrity of the coralline algal skeleton, without need for complex real biological models derived from CT scanning that take ample computer time to construct and analyse. As responses to climate change are species-specific, we are therefore able to create models tailor made to individual species and analyse how they react to future climate change. We have also shown the susceptibility these models have to shear loads rather than compressive loads.

As the oceans are becoming more acidic, with concurrent calcification pressure, it is vital to understand the potential effect of ocean acidification on the skeletons of these habitat forming organisms to infer whether they are able to maintain habitats in the future. As coralline algae are major habitat formers, with the diversity and abundance of species dependent on their structural complexity, weakening of the skeleton under high CO$_2$ conditions will affect the organisms that rely on coralline algae as a habitat with important consequences for marine ecosystems. Our model results, along with bioerosion, rising sea levels (Rhein et al., 2013) and predicted increases in frequency and intensity of storm surges (Knutson et al., 2010) strongly suggest that coralline algae will be under more intense environmental pressure in future CO$_2$ scenarios.

It is important to note that recent long term studies have shown calcifying organisms acclimating to ocean acidification for example cold water corals sustaining growth rates (Form and Riebesell, 2012) or coralline algae decreasing growth rates to maintain cell wall thickness (Ragazzola et al., 2013). The consequence of this sustained growth on the material
properties and structural integrity has not been assessed and poses an open question with regards to their ability to provide habitats in the future.
AUTHOR CONTRIBUTIONS

Leanne Melbourne and Julia Griffin carried out the experiments under the guidance of Daniela Schmidt and Emily Rayfield. Leanne Melbourne prepared the manuscript with contributions from all co-authors.
ACKNOWLEDGEMENTS

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Table 1 Mesh type, number of elements, average von Mises Stress, 95th percentile of (Mises) Stress and Total Strain energy for the different models

<table>
<thead>
<tr>
<th>Model</th>
<th>Mesh type</th>
<th>Number of Elements</th>
<th>Average von Mises Stress (Pa)</th>
<th>95th percentile of von Mises Stress (Pa)</th>
<th>Total Strain Energy (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>422 µatm 2D model</td>
<td>Quad</td>
<td>16368</td>
<td>1.67E+11</td>
<td></td>
<td>8.91E08</td>
</tr>
<tr>
<td>589 µatm 2D model</td>
<td>Quad</td>
<td>1889</td>
<td>5.17E+11</td>
<td></td>
<td>1.27E10</td>
</tr>
<tr>
<td>3D</td>
<td>hexagonal</td>
<td>202304</td>
<td>1.72E+11</td>
<td></td>
<td>1.11E11</td>
</tr>
<tr>
<td>3D*</td>
<td>hexagonal</td>
<td>202304</td>
<td>1.11E+11</td>
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<td>4.70E11</td>
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<tr>
<td>3D (Corridor model)</td>
<td>tetrahedral</td>
<td>2125549</td>
<td>7.46E+10</td>
<td>2.21E+11</td>
<td>1.75E10</td>
</tr>
<tr>
<td>422 µatm compartmentalised (Compartment model)</td>
<td>tetrahedral</td>
<td>3442433</td>
<td>2.75E+10</td>
<td>5.36E+10</td>
<td>4.28E09</td>
</tr>
<tr>
<td>589 µatm compartmentalised (OA model)</td>
<td>tetrahedral</td>
<td>1707673</td>
<td>4.98E+10</td>
<td></td>
<td>8.81E09</td>
</tr>
<tr>
<td>Biologically realistic model (Biological model)</td>
<td>tetrahedral</td>
<td>2106858</td>
<td>2.74E+10</td>
<td>5.63E+10</td>
<td>5.21E9</td>
</tr>
</tbody>
</table>

*Strain energy corrected for calcite volume

Table 2 Percentage volumes of calcite and cavities in the biological model, the corridor and the compartment model.

<table>
<thead>
<tr>
<th></th>
<th>Biological Model</th>
<th>3D Corridor model</th>
<th>3D Compartment model</th>
</tr>
</thead>
<tbody>
<tr>
<td>volume of calcite (µm³)</td>
<td>3.09E+05</td>
<td>1.94E+05</td>
<td>2.87E+05</td>
</tr>
<tr>
<td>volume of cavities (µm³)</td>
<td>1.83E+05</td>
<td>2.60E+05</td>
<td>1.67E+05</td>
</tr>
<tr>
<td>percentage of calcite</td>
<td>63%</td>
<td>43%</td>
<td>63%</td>
</tr>
<tr>
<td>percentage of cavities</td>
<td>37%</td>
<td>57%</td>
<td>37%</td>
</tr>
</tbody>
</table>
Table 3 Average von Mises Stress, 95th percentile of (Mises) Stress and Total Strain energy for the different load types exerted on the biological, compartment and corridor models. Total strain energy for the biological model has been corrected for calcite volume (equation 2). The compartment model under the shear loading type is highlighted to reiterate that the loading setup is different to the biological model under a shear loading type.

<table>
<thead>
<tr>
<th>Model</th>
<th>Loading Type</th>
<th>Average Stress (N µm(^2))</th>
<th>95th Percentile of (Mises) Stress (N µm(^2))</th>
<th>Total Strain Energy (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological</td>
<td>Compressive</td>
<td>2.34E-04</td>
<td>4.08E-04</td>
<td>3.34E+05</td>
</tr>
<tr>
<td></td>
<td>Shear</td>
<td>2.83E-04</td>
<td>6.07E-04</td>
<td>6.11E+05</td>
</tr>
<tr>
<td></td>
<td>Original (Shear + Compressive)</td>
<td>3.49E-04</td>
<td>7.21E-04</td>
<td>9.73E+05</td>
</tr>
<tr>
<td>Compartment</td>
<td>Compressive</td>
<td>2.42E-04</td>
<td>2.96E-04</td>
<td>2.34E+05</td>
</tr>
<tr>
<td></td>
<td>Shear</td>
<td>4.27E-04</td>
<td>1.03E-03</td>
<td>1.63E+06</td>
</tr>
<tr>
<td></td>
<td>Original (Shear + compressive)</td>
<td>4.10E-04</td>
<td>8.25E-04</td>
<td>9.63E+05</td>
</tr>
</tbody>
</table>

Table 4 Average von Mises Stress and Total Strain energy for the comparison of the different material properties in the 2D and 3D corridor models

<table>
<thead>
<tr>
<th></th>
<th>Young’s Modulus (Pa)</th>
<th>Average Von Mises Stress (Pa)</th>
<th>Total Strain energy (J)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcite</td>
<td>3.60E+10</td>
<td>1.67E+11</td>
<td>8.91E+08</td>
</tr>
<tr>
<td>M.edulis -Min</td>
<td>4.39E+10</td>
<td>1.67E+11</td>
<td>7.30E+08</td>
</tr>
<tr>
<td>M.californianus -Min</td>
<td>4.79E+10</td>
<td>1.67E+11</td>
<td>6.69E+08</td>
</tr>
<tr>
<td>M.edulis -Max</td>
<td>7.18E+10</td>
<td>1.67E+11</td>
<td>4.47E+08</td>
</tr>
<tr>
<td>M.californianus -Max</td>
<td>7.93E+10</td>
<td>1.67E+11</td>
<td>4.04E+08</td>
</tr>
<tr>
<td>3D corridor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcite</td>
<td>3.60E+10</td>
<td>7.46E+10</td>
<td>1.75E+10</td>
</tr>
<tr>
<td>M.edulis -Min</td>
<td>4.39E+10</td>
<td>7.46E+10</td>
<td>1.43E+10</td>
</tr>
<tr>
<td>M.californianus -Min</td>
<td>4.79E+10</td>
<td>7.46E+10</td>
<td>1.31E+09</td>
</tr>
<tr>
<td>M.edulis -Max</td>
<td>7.18E+10</td>
<td>7.46E+10</td>
<td>8.77E+09</td>
</tr>
<tr>
<td>M.californianus -Max</td>
<td>7.93E+10</td>
<td>7.46E+10</td>
<td>7.94E+08</td>
</tr>
</tbody>
</table>
Figures

Figure 1

Figure 2
Figure 3

Figure 4
Figure 5

Figure 6
Figure 7

Figure 8
Figure 1: A *Lithothamnion glaciale* specimen in crustose form from Loch Creran, Oban, Scotland; Scale = 1cm.
Figure 2: The effect of elevated CO$_2$ on the *Lithothamnion glaciale* structure. All models are subjected to the same loads and boundary conditions and measurements taken from Ragazzola et al. (2012). Von Mises Stresses are projected onto the 2D Finite element model (a, b) and the 3D compartmentalised models (c, d). Warm colours indicate areas of high stress, while cooler blue colours indicate areas of low stress. Units = Pa.

Figure 3: The four computer derived models; (a) The Corridor model, a simple 3D model; (b) The Compartment model, a compartmentalised model; (c) The Biological model, the biologically realistic model; (d) The OA model, the compartmentalised model under pCO$_2$ conditions simulating the year 2050.

Figure 4: Dimensions used in the 3D corridor model. Units = $\mu$m

Figure 5: Orthoslice projections in the different planes of a rhodolith thallus: a) xy direction; b) xz direction; c) yz direction and d) in 3D format. The purple box highlights where the 80µm x 80µm cube was selected.

Figure 6: Loads and Boundary constraints. All models had the same loads and constraints applied. Loads, representing wave erosion, were applied to the top left hand corner along a strip 40µm wide at the external top left side of the model (yellow surface). Constraints were applied to the bottom right corner (opposite corner to load) along a strip 40µm wide up the right hand side of the model and over the whole of the bottom model (green lines).

Figure 7: The von Mises Stress patterns on the biological model (a, b & c) and the compartment model (d, e & f) in different loading situations; the original (mixture of both shear and compressive loads) (a & d); compressive load (b & e) and the shear load (c & f). The different load situations (pink box) are shown on the biological model as well as the constraints (green boxes). The shear load for the compartment model was set up slightly differently and hence the loading setup is displayed on the model (f). Units = N $\mu$m$^{-2}$. 
Figure 8: (a) 2D vs (b) 3D comparison of the *Lithothamnion glaciale* structure based on Ragazzola et al. (2012) measurements. Units = Pa

Figure 9: Comparison of all 3D models. (a) The 3D corridor model (b) the compartment model and (c) the biological model. Units = Pa.

Figure 10: The inside spheroidal cavities of the biological model