

## Article

# Wanted Dead or Alive: Skeletal Structure Alteration of Cold-Water Coral *Desmophyllum pertusum* (*Lophelia pertusa*) from Anthropogenic Stressors

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**Abstract:** Ocean acidification (OA) has provoked changes in the carbonate saturation state that may alter the formation and structural biomineralisation of calcium carbonate exoskeletons for marine organisms. Biomineral production in organisms such as cold-water corals (CWC) rely on available carbonate in the water column and the ability of the organism to sequester ions from seawater or nutrients for the formation and growth of a skeletal structure. As an important habitat structuring species, it is essential to examine the impact that anthropogenic stressors (i.e., OA and rising seawater temperatures) have on living corals and the structural properties of dead coral skeletons; these are important contributors to the entire reef structure and the stability of CWC mounds. In this study, dead coral skeletons in seawater were exposed to various levels of  $p\text{CO}_2$  and different temperatures over a 12-month period. Nanoindentation was subsequently conducted to assess the structural properties of coral samples' elasticity ( $E$ ) and hardness ( $H$ ), whereas the amount of dissolution was assessed through scanning electron microscopy. Overall, CWC samples exposed to elevated  $p\text{CO}_2$  and temperature show changes in properties which leave them more susceptible to breakage and may in turn negatively impact the formation and stability of CWC mound development.

**Keywords:** biomechanics; biomineralisation; climate change; cold-water coral; porosity; ocean acidification

## 1. Introduction

Cold-water corals (CWCs) are a key component of deep-sea ecosystems [1] and are known to occur in most of the world's oceans except for the Bering Sea and high-latitude Arctic regions [2,3]. *Lophelia pertusa*, formally renamed to *Desmophyllum pertusum* [4], is a well-distributed scleractinian CWC found in most cold-water marine ecosystems globally, and is a dominant species in the northeast Atlantic Ocean [2]. Cold-water corals such as *D. pertusum* require calcium carbonate ( $\text{CaCO}_3$ ) to construct an aragonite skeleton. Such corals represent a deep-sea biogenic source useful for reconstructing past geochemical proxies [5] and ocean circulation, and have the potential to serve as important carbon

sinks [6,7]. Biomineral production in CWCs relies on the availability of  $\text{CaCO}_3$  in the water column and the ability to sequester ions from seawater or nutrients to form and grow a skeletal structure [8,9]. *Desmophyllum pertusum* features thick epithelial and exothelial skeletal components with a clear lamellar growth pattern [10]. Ocean acidification (OA) has provoked changes in the carbonate saturation state [11,12] that may alter the formation and structural biomineralisation of  $\text{CaCO}_3$  exoskeletons for marine organisms [8], and this is concerning for both tropical and CWCs [13]. Little is known about how the microstructure of *D. pertusum* coral skeletons might be altered in such scenarios, but OA has been reported to decrease breaking strength causing the coral skeleton to become more brittle, leading to the disintegration of both live and dead coral skeletons [9,13,14]. If structural integrity of both live and dead corals is compromised due to OA and/or temperature intensification, there is a potential for framework collapse and loss of habitat complexity [9,15].

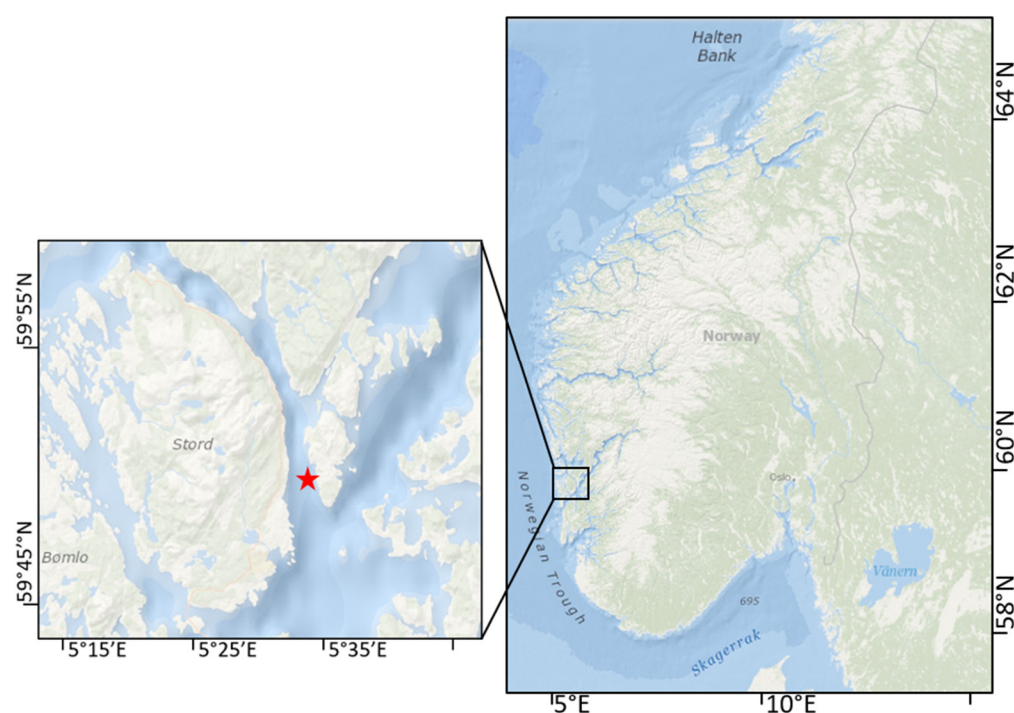
Framework-forming CWCs such as *D. pertusum* produce three-dimensional structures that many organisms use as habitat and nursery grounds, making them biodiversity hotspots [16–22]. Cold-water coral colonies are defined in zones (i.e., macrohabitats) that are typically composed of a base of small coral fragments and sediments to large coral rubble, a matrix of sediment-entrained dead coral fragments and branches, followed by a degradation zone [23] of both live and dead coral framework, and an upper layer of about 20 polyp generations of living corals at the top [2,23]. In the dead coral framework, where live corals are sparse, the highest density of associated organisms is found in relation to the reef framework as a whole [23,24]. For example, with reference to North Atlantic CWC provinces, nearly 75% of the CWC colony at the Mingulay Reef Complex off western Scotland is composed of dead coral framework [25]. In the Belgica mound province in the Porcupine Seabight off southwest Ireland, coral mounds were reported to yield 349 species; much of the live coral framework and dead coral rubble was densely colonised with macrobenthic biodiversity, including calcareous sponges [26]. Megafauna such as bryozoans and macroboring fauna including bivalves, sponges, and polychaetes utilise the sediment-filled cavities of dead coral framework [23]. Furthermore, reef fishes such as the blackbelly rosefish *Helicolenus dactylopterus*, lesser-spotted dogfish *Scyliorhinus canicular*, and blackmouth catshark *Galeus melastomus* rely on CWC frameworks for both hunting and spawning grounds, making these reef habitats more valuable as biodiverse ecosystems [23,27].

As an important habitat structuring species [28], it is essential to examine the impact that OA and rising seawater temperatures have not only on living corals but on the structural properties of dead corals that contribute to the formation and stability of CWC carbonate mounds and reefs [9]. Degradation of dead coral framework that is not supported by protective tissue can occur directly through chemical dissolution, or indirectly due to increased bioerosion [29–31]. Such degradation can result in increased skeletal porosity and the possibility of habitat complexity loss [9]. This study aims to determine if the structural integrity of the dead coral framework will be affected by increasing OA and seawater temperatures, and to assess the capacity of the dead coral framework to withstand these anthropogenic stressors.

## 2. Materials and Methods

Coral samples used here were collected during a Norwegian research expedition aboard the *R.V. Håkon Mosby* from Nakken Reef at a depth of 200–220 m in Norway in 2016 (Figure 1) as part of the “FATE of cold-water corals—drivers of ecosystem change” project funded by the Research Council of Norway. As a follow-on laboratory experiment, this study investigated the impacts of OA and warming on the functioning of CWC ecosystems, including live and dead coral framework, key associated bivalve and sponge species, and changes in nutrient availability to the organisms. The laboratory experiment took place at Austevoll Research Station, Institute of Marine Research, Bergen, Norway, where organisms were observed under various treatments of partial pressure of carbon

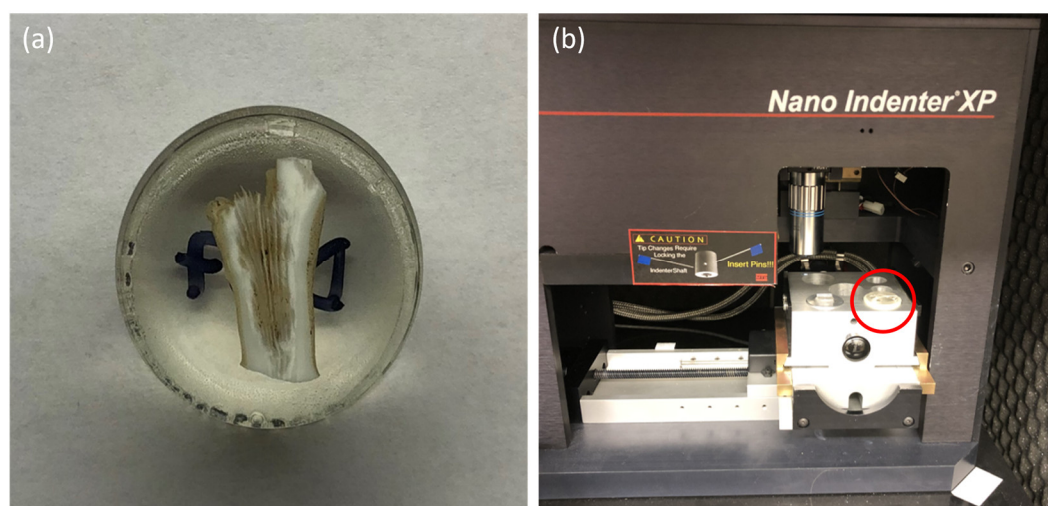
dioxide ( $p\text{CO}_2$ ) and temperature over a 12-month period. The general setup of the OA research facility is a flow-to-waste system consisting of circular fiberglass tanks with slightly conical bottom as described in Andersen et al. [32]. Natural seawater with a salinity of  $\sim 35$  was pumped from 160 m water depth near the station and adjusted in temperature and  $p\text{CO}_2$  in overhead tanks (see details of the temperature and  $p\text{CO}_2$  control in [32]) before being transferred to the experimental tanks. During the FATE project, the following treatments were applied to 45 experimental tanks in total: control or “ambient” at 400  $\mu\text{atm } p\text{CO}_2$  and seawater temperature of 8 °C, “high  $\text{CO}_2$ ” at 1000  $\mu\text{atm } p\text{CO}_2$  and seawater temperature of 8 °C, and “high  $\text{CO}_2+\text{T}$ ” at 1000  $\mu\text{atm } p\text{CO}_2$  and seawater temperature of 11 °C (ambient seabed temperature plus 3 °C increase). In all three temperature and  $p\text{CO}_2$  combinations, three different feeding regimes were applied (low, ambient, and high food). For this study, only ambient food replicates, i.e., samples from unfiltered water tanks, were used.



**Figure 1.** Nakken reef off the coast of Norway (59°49′49.1″ N, 5°33′22″ E; red star), a study area where *Desmophyllum pertusum* samples were collected from a depth of 200–220 m in 2016.

Next, freshly dead coral skeletons free from bioerosion were selected and the soft tissue removed by a 10% diluted bleach solution. Following the 12-month experiment described here, two samples were selected at random from each experimental tank (i.e., ambient, high  $\text{CO}_2$ , and high  $\text{CO}_2+\text{T}$ ) for a total of six samples. Samples were cleaned and checked for integrity, taking note that no degradation due to biological factors was present. Samples were prepared by cutting coral branches at an aspect ratio as close to 1:1 as possible. As coral branches are not uniform and may be tapered, an ideal geometry cannot be achieved. Samples were individually mounted in epoxy resin (Struers EpoFix Kit) and cured in an oven at 30 °C for 24 h. Once cured, samples were cut using a Buehler IsoMet 1000 Precision Cutter (Lake Bluff, IL, USA) low-speed saw to expose a hollow section on the top surface of the mounts. The exposed surfaces of the mounts were further polished (Struers LaboPol-21 polisher with diamond suspension) to a 1  $\mu\text{m}$  finish (Figure 2a). This process creates a planar and highly polished surface suitable for indentation across the section from the outside surface toward the inner surface. An MTS® Nano Indenter® XP (Eden Prairie, MN, USA) fitted with a diamond tip (Figure 2b) was used to assess coral

properties to establish elasticity (i.e., stiffness) using Young's modulus ( $E$ ) and coral hardness using Vickers hardness ( $H$ ). Average  $E$  and  $H$  values were assessed at a nanoindentation depth range of 2000 nm along the solid white areas of the coral for a single coral fragment (data file available in Supplementary Materials). In this study, we consider elasticity as the ability of the sample to resist deformation and return to its original state when a force being applied is removed, while hardness is the threshold to which the sample would become permanently deformed [9]. Six samples of *D. pertusum* were analysed with a total of 142 indentations.

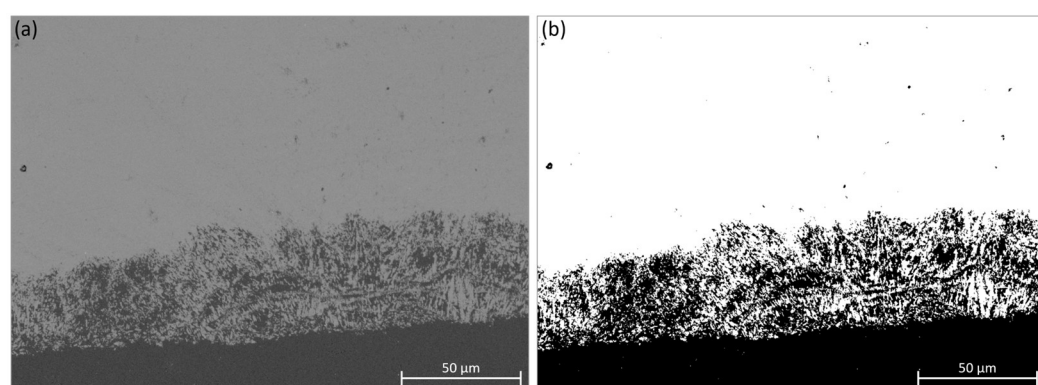


**Figure 2.** Preparation of coral sample (a) *Desmophyllum pertusum* set in resin, cut and polished with exposed skeleton prior to nanoindentation, and (b) a prepared coral sample in the mount (red circle) of the MTS Nano Indenter® XP (Dublin, Ireland).

Following nanoindentation, backscatter scanning electron (BSE) images were captured using the Tescan TIGER MIRA3 (Brno–Kohoutovice, Czech Republic) scanning electron microscope (SEM) at the Centre for Microscopy and Analysis at Trinity College Dublin. Prior to imaging, coral samples were carbon coated to avoid excess charged particles. Images were taken at 5kV with a scanning speed of 6 and were used to detect visual changes in porosity.

A one-way analysis of variance (ANOVA) was run using JMP® Statistical Discovery Software [33] to determine if there were statistically significant differences between samples taken from each of the three experimental tank treatments (i.e., ambient, high CO<sub>2</sub>, and high CO<sub>2</sub>+T) for  $E$  and  $H$  [8]. Due to the relatively small sample size, a post hoc Tukey's honestly significant difference (HSD) was run to determine how the treatments differed from one another by a pairwise comparison and Shapiro–Wilk (W) was run to check for normality. Porosity was obtained by analysing SEM images along the outer coral wall using ImageJ software [34]. Porosity was calculated from the percentage of the outer wall where visible porosity was observed in the binary image as shown in Figure 3 [35].





**Figure 3.** Examples of image transformation from (a) scanning electron microscope image to (b) binary image for porosity calculation using ImageJ [34].

### 3. Results

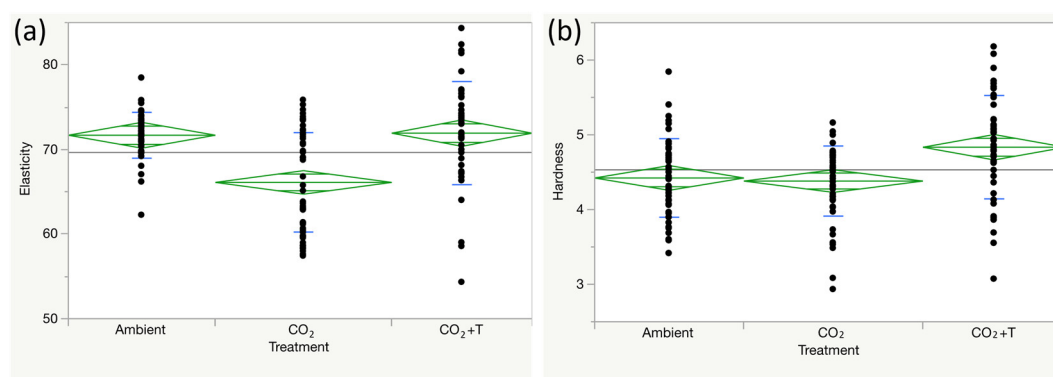
Both nanoindentation analyses and SEM imaging indicate changes in the structural integrity of the coral skeleton at certain elevated CO<sub>2</sub> and temperature conditions (Table 1).

**Table 1.** Analysis of Young's Modulus ( $E$ ) and Vickers Hardness ( $H$ ) mean and standard deviation of dead *Desmophyllum pertusum* samples exposed to various experimental conditions.

Condition	Indentation (n)	$E$ Mean (GPa)	$E$ Std. Dev.	$H$ Mean (GPa)	$H$ Std. Dev.
Ambient	45	71.7	2.7	4.4	0.5
High CO <sub>2</sub>	54	66.1	5.9	4.4	0.5
High CO <sub>2</sub> +T	43	71.9	6.1	4.8	0.7

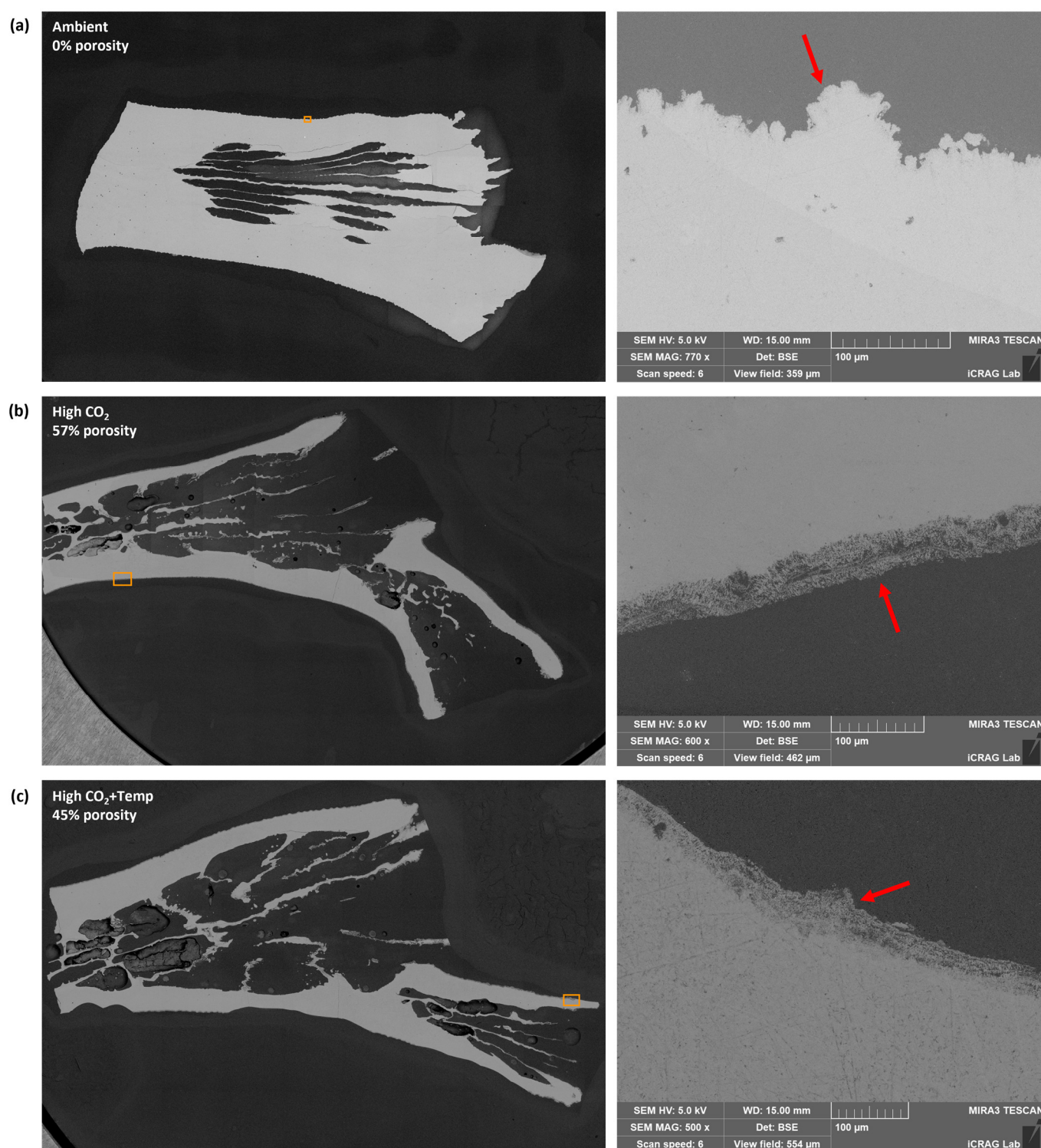
A one-way ANOVA of indentation data showed a statistically significant difference for  $E$  ( $F = 20.328$ ;  $p < 0.0001$ ). Elasticity ( $E$ ) significantly decreased ( $p < 0.0001$ ) in high CO<sub>2</sub> samples, while there was no significant difference between ambient and high CO<sub>2</sub>+T samples (Figure 4a). A significant difference was observed between ambient and high CO<sub>2</sub> samples ( $p < 0.0001$ ) and between high CO<sub>2</sub> and high CO<sub>2</sub>+T samples ( $p < 0.0001$ ), suggesting that CO<sub>2</sub> influences coral elasticity to an extent that caused a structural change in the coral, such as increased porosity (Shapiro–Wilk test for normality passed,  $p < W = 0.45$ ).

A statistically significant difference was analysed for  $H$  ( $F = 8.989$ ;  $p < 0.0002$ ); although it was less significant when compared to  $E$ . Hardness ( $H$ ) significantly increased ( $p < 0.0001$  for ambient;  $p < 0.0004$  for high CO<sub>2</sub>) in high CO<sub>2</sub>+T samples (Figure 4b). There was no significant difference between ambient and high CO<sub>2</sub> samples, but a statistical difference between ambient and high CO<sub>2</sub>+T ( $p < 0.0001$ ) and between high CO<sub>2</sub> and high CO<sub>2</sub>+T ( $p < 0.0008$ ) was observed, suggesting that combined high CO<sub>2</sub>+T influences coral hardness to an extent (Shapiro–Wilk test for normality passed,  $p < W = 0.11$ ).



**Figure 4.** Statistical analyses (one-way analysis of variance) of indentation data for *Desmophyllum pertusum* (a) elasticity and (b) hardness from ambient, high CO<sub>2</sub>, and high CO<sub>2</sub>+T conditions. High CO<sub>2</sub> was significantly different for elasticity and high CO<sub>2</sub>+T was statistically different for hardness. The grey line running across each figure is the mean of the response. Refer to Table 1 for number of indentations for each of the three experimental treatments.

The presence of porosity changes along the outer coral wall can have a large effect that may lead to the coral bending and twisting as water flows around it; surface defects have a strong effect in reducing the strength, especially in a brittle material such as CaCO<sub>3</sub>. Scanning electron microscopy (SEM) images were used to evaluate the microstructure and analyse relative changes in the porosity of the dead coral samples. Visible degradation of the outer coral wall (Figure 5) was observed in both coral samples exposed to high CO<sub>2</sub> alone. No degradation was observed in SEM images of the ambient samples.



**Figure 5.** Scanning electron microscopy images for *Desmophyllum pertusum* samples showing visible material alterations of the outer coral walls for (a) ambient, (b) high CO<sub>2</sub>, and (c) high CO<sub>2</sub>+T samples. Full coral sample images are on the left and the orange inset boxes display zoomed images on the right. Red arrows represent the area of the outer wall where changes in porosity were calculated.

#### 4. Discussion

Live CWCs are capable of producing calcifying extracellular mucus substances which are considered important in the formation of the thick epithelial skeletal tissue [10]. Once mineralised, the coral skeleton plays an important role in providing a framework for mound formation and stability. From this study, we determined that the structural

integrity of the coral skeleton is compromised at certain elevated  $p\text{CO}_2$  and seawater temperature conditions. Elasticity significantly decreased in samples exposed to the high  $\text{CO}_2$  variable, meaning that with increased  $\text{CO}_2$  conditions, the  $\text{CaCO}_3$  coral skeleton becomes more brittle. Hardness significantly increased in samples exposed to high  $\text{CO}_2+\text{T}$ , as shown in Figure 4, leading us to postulate that skeletal deformation resulting from increased hardness may occur at a later stage with longer running experiments, or in natural systems. Since we do not see such changes in samples exposed to high  $\text{CO}_2$  alone, we suspect that coral hardness is increased with elevated temperatures. Overall, we observe that increasing  $p\text{CO}_2$  and temperature caused the partial dissolution of the biomineralised skeleton. At higher temperatures, some of the dissolved material may reprecipitate back into the coral which could explain  $H$  increasing with a temperature increase [36,37].

Studies analysing the structural properties of biomineralisation of calcifying organisms are relatively novel, and very few have been conducted on CWCs and bivalves. Hennige et al. [14] conducted an experiment on *D. pertusum* growth and respiration from the Mingulay Reef Complex to various increased  $\text{CO}_2$  levels and temperature conditions over a 12-month period. They did not find significant impacts of OA and warming on respiration or growth but postulated that to some extent *D. pertusum* can acclimatise physiologically. However, there was a negative correlation between increasing  $\text{CO}_2$  levels and breaking strength of roughly 20–30% weakness in areas where coral tissue decreased and left the skeleton exposed. From this, they concluded that the exposed reef bases will become less effective ‘load-bearers’ and will be more susceptible to bioerosion and mechanical damage by the year 2100. In our study, although  $H$  is not changed in high  $\text{CO}_2$  samples, the decrease in  $E$  would support the findings by Hennige et al. [14] regarding reduced structural integrity. Hennige et al. [9] further examined the same coral samples as their earlier study, focusing on the porosity of the coral skeleton. Live *D. pertusum* displayed no loss in porosity except in areas where tissue loss occurred; yet, dead corals had completely increased skeletal porosity of both the inner and outer walls when exposed to increased  $\text{CO}_2$ . ‘Coralporosis’, as Hennige et al. [9] refer to in their study, as the loss of skeletal strength and/or density quite similar to osteoporosis in bones could occur from decreased structural integrity, leading to framework foundation loss, and ultimately resulting in large-scale habitat loss or prevention of coral mound growth. Our results are similar to these findings of acidification-induced skeletal porosity along the outer walls of dead corals (Figure 5), and further, they validate the mathematical modelling presented by Hennige et al. [9] for stress due to bending from water flow. Although similar in results, the significant differences we present here from increased  $p\text{CO}_2$  and temperature could be explained by the difference in scenarios of the previous studies (i.e., the experimental setup of our study for high  $\text{CO}_2+\text{T}$  was at 1000  $\mu\text{atm}$  at 11 °C versus 750  $\mu\text{atm}$  at 12 °C from Hennige et al. [14]).

Wolfram et al. [13] further analysed live coral samples from Hennige et al. [9] with regard to the mechanisms of structural changes under increasing porosity and dissolution underpinning the laboratory experiments with mathematical and computational models. They revealed a compressive strength (462 MPa) and stiffness (45 to 67 GPa) of the skeletal material that is 10 times stronger than concrete. Surprisingly, CWCs seem to retain their skeletal strength despite the loss of stiffness under future ocean conditions. However, their models resulted in a significant increase in coral habitat crumbling from small porosity increases, concluding that OA affects dead coral skeletons through dissolution and porosity, leading to a decrease in thickness of the skeletal wall and ultimately a detriment to the fragility of the exposed coral skeleton [13]. This is further supported by our study showing decreased  $E$  (stiffness) and increased porosity under future elevated  $\text{CO}_2$  conditions. However, again, the increased level of  $H$  in high  $\text{CO}_2+\text{T}$  we present here contrasts the findings of Wolfram et al. [13], which could again be explained by different crystal arrangements in the corals used in our study.

Similar studies on the biomineralisation and material properties of the common blue mussel *Mytilus edulis* were conducted by Fitzer et al. [8,38]. Fitzer et al. [38] analysed



mussel biomineralisation to determine if there was a present OA threshold or tipping point. Their results showed that shell growth continued with increased  $p\text{CO}_2$ ; yet, the microstructure displayed crystallographic disorientation similar to findings in Hennige et al. [14] for the CWC *D. pertusum*. Following that study, Fitzner et al. [8] analysed *E* and *H* for *M. edulis* and found that under increased OA conditions, the outer calcite shell was more brittle and the inner aragonite shell was softer and less stiff. This showed that even though calcite is the more stable polymorph of  $\text{CaCO}_3$  [39], similar results were found in *D. pertusum* with its aragonite skeleton. However, the significance of the  $p\text{CO}_2$  impact was reduced in *M. edulis* when a seawater temperature variable was introduced, leading to the conclusion that there may be a threshold that mussels can withstand. Our study presented here displayed similar results of significant increases in *E* for elevated  $p\text{CO}_2$  compared with ambient conditions, as well as a reduced impact with the addition of higher seawater temperature. Thus, projected climate change will likely have an impact on shell structure and properties to some extent, in both calcite and aragonite-calcifying marine organisms.

In addition, bioerosion is found to be accelerated under OA both in tropical and CWC reefs, where a simultaneous increase in temperature did not counteract the impact of acidification [29,31]. Accelerated bioerosion will further lead to the degradation and weakening of coral reef frameworks, which will have implications for the biodiversity of these ecosystems. From our results and similar studies, we can therefore conclude that there is concern regarding skeletal structure integrity, possible framework instability, a reduced load-bearing capacity, and susceptibility to greater than normal bioerosion and mechanical damage for CWC reefs in the future if acidification and seawater temperatures continue to increase in our oceans.

## 5. Conclusions

The shift from a complex habitat comprised of live and dead corals to a less complex habitat comprised of live corals alone is a major threat to CWC ecosystem biodiversity [9]. As previously stated, dead corals provide a suitable framework for macrohabitats, leading to enhanced metabolic activity, high oxygen consumption, and mineralisation of organic matter [40,41] which is vital to reefs as a whole. A continued decrease in coral skeletal structure will ultimately result in a large-scale habitat loss and prevention of future coral mound growth. Further long-term studies and microscopy imaging would provide useful insight to examine the change in the porosity of dead coral framework. Our results suggest that increased OA and rising seawater temperatures will have an impact on the structural integrity of CWC reefs, especially on the outer walls of dead corals. This study highlights the need to further validate CWC skeletal structure alterations on a larger scale with an expanded range of conditions, along with analyses of possible changes in mineralisation and quantifiable reprecipitation from increased temperature through specific laboratory experiments. Modelling such changes may provide a better understanding of how and when these projected impacts will occur.

**Supplementary Materials:** The following supporting information can be downloaded at [www.mdpi.com/article/10.3390/oceans4010006/s1](http://www.mdpi.com/article/10.3390/oceans4010006/s1), Spreadsheet S1.

**Author Contributions:** Conceptualization, E.T.K.; data curation, E.T.K. and P.J.O.; formal analysis, E.T.K. and P.J.O.; funding acquisition, J.V.B. and Q.G.C.; investigation, E.T.K. and J.V.B.; methodology, E.T.K., J.V.B., D.A.H., D.T. and P.J.O.; resources, D.A.H., D.T. and Q.G.C.; software, E.T.K. and P.J.O.; supervision, Q.G.C.; validation, E.T.K. and Q.G.C.; visualization, E.T.K., J.V.B. and Q.G.C.; writing—original draft, E.T.K.; writing—review and editing, E.T.K., J.V.B., D.A.H., D.T. and Q.G.C. All authors have read and agreed to the published version of the manuscript.

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