Title: Assessing the living and dead proportions of cold-water coral colonies: implications for deep-water Marine Protected Area monitoring in a Changing Ocean

Johanne Vad[1,2*], Covadonga Orejas[3], Juan Moreno-Navas, Helen S Findlay[4], J Murray Roberts[2, 5*]

*Corresponding author: jv63@hw.ac.uk; murray.roberts@ed.ac.uk

1 Heriot-Watt University, School of Engineering Geoscience Infrastructure and Society, Edinburgh, Scotland EH14 4AS, United Kingdom

2 University of Edinburgh, School of Geoscience, Grant Institute, Edinburgh, Scotland EH9 3FE, United Kingdom

3 Instituto Español de Oceanografía (IEO), Centro Oceanográfico de Baleares (COB), Moll de Ponent s/n, 07015 Palma de Mallorca, Islas Baleares, Spain.

4 Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth, PL1 3DH, United Kingdom

5 Center for Marine Science, University of North Carolina Wilmington, 601 S. College Road, Wilmington, NC 28403-5928, United States of America

Short title: cold-water coral growth assessment

Abstract

Coral growth patterns result from an interplay of coral biology and skeletal structure modulated by environmental controls. In this study, colony size and layering in the cold-water coral (CWC) Lophelia pertusa *(Linnaeus, 1758) were measured using video footage from Remotely Operated Vehicle (ROV) transects conducted at the inshore Mingulay Reef Complex (MRC) and at the offshore PISCES site (Rockall Bank) in the NE Atlantic. The main goal of this paper was to explore the development of a simple method to quantify coral growth, and its potential application as an assessment tool of the health of these remote habitats. Eighteen colonies were selected and whole colony and dead/living layer size were measured. Live to dead layer ratios for each colony were~~as~~ then determined and analysed. Age of each colony was estimated using a previously determined growth rate.*

Our paper shows that:

(1) two distinct morphotypes can be described: at the MRC, colonies displayed a 'cauliflower-shaped' morphotype whereas at the PISCES site, colonies presented a more flattened 'bush-shaped' morphotype.

(2) Living layer size was positively correlated with whole colony size

(3) Live to dead layer ratio was negatively correlated to whole colony size

(4) Live to dead layer ratio never exceeded 0.27.

These results suggest that as a colony develops and its growth rate slows down, the proportion of living polyps in the colony decreases. Furthermore, at least 73% of L. pertusa *colonies are composed of exposed dead coral skeleton, vulnerable to ocean acidification and the associated shallowing of the Aragonite Saturation Horizon, with significant implications for future deep-sea reef framework integrity. The clear visual contrast between pale living and darker dead portions of the colonies also gives a new way by which they can be visually monitored over time. The increased use of marine autonomous survey vehicles offers an*

Commented [U1]: It can be argued that skeletal structure is a function of coral biology. I would delete skeletal structure.

Commented [U2]: Suggest 'proportion of live and dead skeleton'

important new platform from which such a monitoring technique could be applied to monitoring deep-water marine protected areas monitoring in the future.

Commented [U3]: surveying

Keywords

Cold-water corals, *Lophelia pertusa*, Mingulay Reef Complex, PISCES site, Rockall Bank, colony size, dead framework, ocean acidification, monitoring deep-water Marine Protected Areas

INTRODUCTION

Cold-water corals (CWC) form complex 3-dimensional reef frameworks maintaining high biodiversity hotspots (e.g. Freiwald 2002; Roberts *et al.* 2009; Henry *et al.* 2010), but several anthropogenic activities are putting them at risk. Fisheries, oil and gas extraction as well as the effects of global change, including ocean acidification (OA), are impacting these important benthic communities (e.g. Roberts *et al.* 2006; Hall-Spencer *et al.* 2008; Hennige *et al.* 2015). One of the factors that clearly defines the resilience of such fragile benthic communities to natural and anthropogenic impacts, as well as the population dynamics of clonal species such as corals, are~~is~~ their longevity, growth rate and growth pattern (Hughes 1987), but, in comparison with their tropical counterparts, azooxanthellate CWC still remain less known and much less studied due to the difficulties in accessing their remote deep-sea locations. Coral growth is controlled by a range of environmental factors. In deep waters (> 100 m), local hydrodynamics and food supply (Mienis *et al.* 2007), as well as temperature (Thresher 2009) play a central role for survival and growth of CWC. For many years these factors have also been known to modify tropical coral phenotype; for example, branching tropical corals tend to become less robust with depth (Barnes 1973) but less is known about their impact on CWC phenotypes. Over the last 15 years improvements in aquaria cultivation (e.g. Roberts & Anderson 2002; Olariaga *et al.* 2009) and in the growing use of high resolution visual surveys from Remotely Operated Vehicles (ROV), have allowed great strides to be made in our understanding of these previously hidden ecosystems. Video footage and still images obtained with ROVs have become powerful non-destructive approaches to study several aspects of CWCs and the communities they support including their occurrence, density and geographic distribution (e.g. Orejas *et al.* 2009; Arnaud-Haond *et al.* 2015), bathymetric distribution, coral size classes and orientation (Gori *et al.* 2013) and relationship with associated species (Purser *et al.* 2013).

Commented [U4]: some species of CWC

Commented [U5]: use either high biodiversity or biodiversity hotspots – don't need both

Commented [U6]: suggest using 'threatening' and including deep sea mining. OA is more of a threat than a current impact and so is mining.

Commented [U7]: Very long sentence – split into two sentenes

Commented [U8]: Food supply? Shallow corals have different 'food' source. Suggest you modify to energy supply.

Commented [U9]: Suggest 'influence'

Our knowledge of CWC growth rates has also dramatically improved thanks to experimental studies in aquaria (e.g. Orejas *et al.* 2008, 2011a, b; Brooke and Young 2009; Lartaud *et al.* 2013) and field measurements on man-made structures (Gass and Roberts 2006, 2010; Larcom *et al.* 2014). Moreover, data both on abiotic parameters and from video and photographic records can now allow morphological patterns and colony biometrics to be described, quantified and related to abiotic environmental data. This synergistic approach linking colony morphology and size to environmental parameters is needed to gain deeper understanding of the relationship between those drivers and CWC growth. These are the critical first steps necessary if field monitoring programmes are to be established to understand and record the implications of global change on CWC habitats.

Tto date most laboratory CWC studies have workd on *Lophelia pertusa* (Linnaeus, 1758). Several studies show the degree of adaptation of this species to temperature changes (e.g. Dodds *et al.* 2007; Naumann *et al.* 2014), and in recent years, much effort has been made to understand the effects of ocean acidification on *L. pertusa* growth (e.g. Form and Riebesell 2011; McCulloch *et al.* 2012; Maier *et al.* 2013; Movilla *et al.* 2014; Hennige *et al.* 2015) and the carbonate chemistry of the environments where it occurs (e.g. Findlay *et al.* 2014). The recent results from studies performed using *L. pertusa* from the Mingulay Reef Complex (Roberts *et al.* 2005, 2009), indicated that this species was fairly resilient under OA scenarios under short timescales (Maier *et al.* 2013; Hennige *et al.* 2014a). However, over longer experimental time periods of a year, biomineralisation processes in *L. pertusa* changed inducing modifications in polyp morphology and making the skeleton more fragile, with additional evidence that dead portions of the skeleton not covered by living coral tissue were particularly vulnerable (Hennige *et al.* 2015). Thus understanding the proportions of live and dead coral during field surveys is an essential prerequisite of any long-term monitoring programme to follow CWC framework reefs over time.

Commented [U10]: Delete – not all of these studies were in aquaria.

Commented [U11]: Add Brooke et al 2013.
Brooke **S,** SW Ross, JM Bane, HE Seim, CM Young (2013) Temperature tolerance of the deep-sea coral *Lophelia pertusa* from the southeastern United States. Deep Sea Research II. 92:240-248

In this paper, we explore a new approach to assess *L. pertusa* colony size, and the proportion of live and dead coral in each colony by using opportunistic measurements from high definition video footage recorded from two sites in the NE Atlantic, one inshore and one offshore. This opportunistic study was completed with the footage available from the 2012 Changing Oceans Expedition (RRS *James Cook* cruise 073) and revealed: (1) that distinct colony morphotypes dominate each study site and (2) that both morphotypes were predominantly composed of dead coral with smaller proportions of live coral polyps found in all colonies analysed. Based on these preliminary results, we explore the potential applications of this coral growth quantification as an assessment tool to determine the health and conservation status of deep-sea reefs.

MATERIAL & METHODS

Research area

The Mingulay Reef Complex (MRC) is located in the Sea of the Hebrides between the uninhabited island of Mingulay and the west coast of Scotland (Fig. 1). Within the MRC, Mingulay Reef Area 1 (MR) constitutes two asymmetric east-west oriented ridges ~~respectively~~ 1.5 and 2.3 km long respectively, separated by an approximately 700 m wide gap (Fig. 1). The so-called Banana reef (BR) to the southeast of MR, is formed by a thin 2.5 km-long ridge (Fig. 1) (Roberts *et al.* 2009; Duineveld *et al.* 2012). The coral colonies forming the MRC grow preferentially on the topographic highs created by the flanks and crests of ridges formed by dolorite sills (MR) and igneous intrusions (BR) that outcrop at the seabed (Roberts *et al.* 2009). Video transects used in this study revealed a high coral cover but our analyses were at times constrained by poor visibility at this site in the post-spring bloom time period when the surveys were carried (RRS *James Cook* cruise 073, Roberts *et al.* 2013).

Comprehensive information on abiotic factors were available throughout MRC: depth at

Commented [U12]: Depths?

Commented [U13]: What about temperature? It's possibly the most important abiotic influence on growth.

and site carbonate chemistry were known thanks to successive surveys starting in 2003 (Roberts *et al.* 2005, 2009; Davies *et al.* 2009; Findlay *et al.* 2013) and average current speed, current speed standard deviation, maximum current speed, aspect, slope as well as rugosity were extracted from a high resolution 3D hydrodynamic model with 100 m spatial resolution (Moreno-Navas *et al.* 2014). These seabed terrain variables were calculated with a spatial resolution of 3 m using ArcGis 9.2 with ESRI spatial analysis extension (Moreno-Navas *et al.* 2014).

The other study location was the PISCES site on the Rockall Bank. The offshore PISCES site has been less intensively studied than the MRC although it was first described by Wilson (1979) during research submersible dives using PISCES III in 1973. In this area *L. pertusa* shows a discontinuous patchy distribution of 'Wilson rings', mostly at depths of 220-350 m where coral colonies grow on the flanks of Pleistocene iceberg ploughmarks (Wilson 1979). Video transects recorded here also covered extensive off-reef habitats, illustrating the sparsity of the coral cover at the PISCES site in comparison with MRC (RRS *James Cook* cruise 073, Roberts *et al.* 2013).

Commented [U14]: Was there no environmental or bathymetric data for this site?

Sampling and video processing

In the present study, video surveys were conducted at MRC and PISCES, during the 2012 Changing Oceans Expedition (RRS *James Cook* cruise 073, Roberts et al. 2013) carried out through the Natural Environment Research Council's UK Ocean Acidification research program (UKOA, NERC). The cruise took place in May-June 2012 and high definition video footage was recorded with the *Holland-1* remotely operated vehicle (ROV), from the Irish Marine Institute (Galway). ROV position was recorded by an ultra-short-baseline system (USBL) underwater positioning system.

A total of 9 video surveys, 7 from MRC (6 from MR and 1 from BR) and 2 from

156 PISCES were used in this study. From these dives, all colonies (1) visibly distinctive from
157 others on the video footage and close enough to the ROV for precise measurements (within 1
158 m) and (2) displaying a clearly visible separation between the darker dead and the brighter
159 white/orange living layers of the coral colony were selected and still images of those colonies
160 were extracted from the videos (Fig. 2). In this study, a *L. pertusa* colony refers to a
161 distinctive coral sub-entity of the reef. We recognise that skeletal fusion in *L. pertusa* is
162 common (Hennige *et al.* 2014b) and therefore do not use the word colony to imply any
163 genetic differentiation.
164 Universal time codes (UTC) were not embedded in the high definition footage but were
165 available via low definition images recorded by three additional ROV cameras.
166 Synchronisation of the two video records enabled the time each colony was filmed to be
167 extracted and from this their precise positions (Universal Transverse Mercator, UTM) were
168 logged using the OFOP (Ocean Floor Observation Protocol, Huetten and Greinert 2008)
169 navigation system output. Videos were replayed and processed using iMovie (Apple Inc.).
170
171 **Colony measurements and age estimation**
172 The *Holland-1* ROV was equipped with two laser scale pointers separated by 100 mm
173 which were used to assess overall coral colony size and the thicknesses of the dead and living
174 layers within each colony. On rare occasions, the two laser beams were not visible because of
175 high water turbidity. In these cases, it was possible to estimate colony sizes using the known
176 dimensions (400 mm width) of a bio-box sampling unit mounted immediately adjacent to the
177 corals in the field of view at the front of the ROV (see figure 2a). To limit perspective errors
178 only colonies immediately adjacent to the bio-box were measured in this way.
179 Each colony image was processed using the free software ImageJ (Rasband, W.S.,
180 ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA,

181 http://imagej.nih.gov/ij/, 1997-2014). For each image, the size of the whole colony (from base
182 to top) and of each layer (dead and living part) was measured at five different points on each
183 colony in order to best catch intra-individual variability. To perform these measurements, the
184 growth direction of the coral branches was followed (Fig. 2). Thus, after image processing,
185 five measurements per colony were available for the whole colony length as well as for the
186 dead and the living layer (Fig. 2). The 'living layer length : whole colony length' ratio
187 (LL:WC ratio) was also calculated for each colony dividing the length of the living layer by
188 the length of the whole colony.
189 Age for each colony was determined using a growth rate estimation of 26 ± 5mm yr^{-1}
190 (Gass and Roberts 2006). Although several studies have assessed different *L. pertusa* growth
191 rate using *in-situ* measurement, coral staining and aquaria approaches (Duncan 1877; Dons
192 1944; Orejas *et al.* 2008, 2011b; Brooke *et al.* 2009; Lartaud *et al.* 2012), the Gass and
193 Roberts (2006) estimation is based on *in situ* colony observation over time in the North Sea
194 using ROV recorded video footage and therefore gives the closest match geographically and
195 in terms of water depth as well as technologically to this study.
196
197 **Numerical and statistical analysis**
198 Average values of layer lengths, whole colony lengths and LL : WC ratios were
199 determined for each colony. Two-sided two samples Wilcoxon tests were then performed for
200 each metric. Further statistical analysis was also carried out by calculating Spearman
201 correlation factors and p-values between the whole colony size and the living layer thickness
202 as well as the LL : WC ratio. For these calculations, all of the measurements were used (18
203 colonies x 5 replicates for each colony). Moreover, for corals at the MRC, average current
204 speed, current speed standard deviation, current speed maximum as well as depth and aspect
205 (facing gradient of the seabed), slope (gradient) and rugosity were available using output from

the model described by Moreno-Navas *et al.* (2014). Spearman correlation coefficient and p-value were respectively calculated between these abiotic factors and each layer size as well as -log transformed ratio. All statistical handling was performed with the free software R (R Development Core Team 2011).

Commented [U15]: Explain this a little more – did you correlate each coral factor with each of the 7 factors separately? This seems rather cumbersome – I recommend looking at some statistical approach that will look at hierarchical effects. Were there no comparable data for Pisces?

RESULTS

***Lophelia pertusa* colonies morphotypes from MRC and PISCES**

In total 18 colonies from nine transects varying from 63 to 1,865 m in length, displayed a clear live/dead layer separation and could thus be used in the analysis (table 1, Fig. 1). Fourteen of these colonies were located at MRC (colonies 1 to 14) and the 4 remaining were situated in PISCES (colonies 15 to 18) (table 1).

Differences in colony morphology were identified between the two areas: *L. pertusa* colonies at MRC displayed a spherical "cauliflower" shape (*sensu* Freiwald et al. 1999; Rogers 2004; Orejas et al. 2009), resulting from a multidirectional growth (Fig. 2a). On the contrary, *L. pertusa* colonies at PISCES were less abundant then in MR and more flattened and horizontally planar in their shape, emerging from a horizontal growth (Fig. 2b). Wilson (1979) called the PISCES morphotype 'bush-shaped' and these colonies displayed a less compact shape than those at MRC with some portions of the colonies not covered by living polyps (Fig. 2b).

Colony size and layer length estimation

Overall *L. pertusa* whole colony size ranged from 324 ± 43 mm (Colony 2, MRC) to 1,344 ± 115 mm (Colony 13, MRC) (Table 2, Fig. 3). Therefore, the estimated ages of the colonies observed in this study ranged from 12.9 ± 3.1 (colony 2, MRC) to 53.7 ± 11.7 years old (colony 13, MRC) (Table 2).

231 The living layer size stayed relatively stable: varying from a minimum of 48 ± 13 mm
232 (colony 2, MRC) to a maximum of 260 ± 10 mm (colony 5, MRC) (Table 2, Fig.3). In
233 contrast to the living layer, the dead layer length varied notably between colonies, accounting
234 for the variability of the whole colony size described above. Colony 13 showed the longest
235 dead layer (1,208 ± 124 mm) whereas the smallest dead layer (207 ± 26 mm) was measured in
236 colony 9 (Table 2, Fig.3).
237 Difference in layer sizes and whole colony sizes between sites was not observed. However,
238 living and dead layer length variations were overall less notable at the PISCES site (Table 2,
239 Fig.3).
240 In all the colonies measured in this study, the living layer never exceeded one fourth of
241 the whole colony size, resulting in LL : WC ratios ranging from 0.10 ± 0.02 (colony 13,
242 MRC) to 0.27 ± 0.02 (colony 5, MRC) (Table 2). The LL : WC ratio variation for colonies
243 from PISCES was very narrow with minimum values of 0.16 ± 0.02 (colony 16) to maximal
244 of 0.18 ± 0.03 (colony 15 and 18) (Table 2).
245
246 **Statistical analysis**
247 All two-sided two samples Wilcoxon tests (on layer lengths, whole colony sizes and -
248 Log transformed LL : WC ratio) produced non-significant p-values (ranging from 0.1515 to
249 0.915) showing no statistically significant differences between the colonies from PISCES and
250 MRC sites (Table 3) for all the metrics measured here.
251 Spearman correlation tests however revealed a significant positive correlation (p-value=
252 $1.1e^{-11}$, ρ=0.63) between whole colony size and living layer size and a significant negative
253 correlation (p-value=$4.4e^{-04}$, ρ=-0.35) between whole colony size and LL : WC ratio (Fig. 4).
254 Correlation coefficients between living and dead layer thicknesses and abiotic factors
255 (depth, average current speed, aspect, slope and rugosity) were overall low and not significant

Commented [U16]: largest

Commented [U17]: if you have temperature for the sites, it should be included in the analysis. If not, discuss how temperature and other factors such as food, which also was not measured, would probably have a greater influence on growth than the factors used in the analysis.

as all p-values varied between 0.36 and 0.98.

DISCUSSION

***Lophelia pertusa* morphotypes and the influence of environmental factors**

This study reveals the presence of two distinct *L. pertusa* colony morphologies at MRC ("cauliflower") and PISCES area ("bush-shaped"). Different morphotypes have already been documented in tropical scleractinian corals, which are also known to display several growth forms probably due to an interplay between factors including: (1) genetic differences between individuals or populations (Willis and Ayre 1985), (2) different influences of abiotic factors such as depth (Barnes 1973), water movement or turbidity (Miller 1995; Dullo 2005), (3) a synergy of several abiotic factors (e.g. Foster 1979; Smith *et al.* 2007) as well as a combination of genetic and abiotic features (e.g. Via and Lande 1985). Similar observations concerning CWC colony plasticity have been so far reported by Freiwald (2002), Orejas *et al.* (2009) and Gori *et al.* (2013) and unravelling environmental conditions leading to the differentiation in *L. pertusa* colony shape could lead to the use of morphotypes as a bio-indicator of environmental conditions, an idea already suggested by Grigg (1972) for gorgonians.

The different morphologies found in the two sites could be due to differences in environmental conditions experienced by the colonies at these locations: MRC and PISCES are reef structures and coral patches respectively (Wilson 1979; Roberts *et al.* 2009). Those two sites also display different depth ranges: the reefs studied in the MRC are located between 100 – 137 m depth whereas the PISCES coral patches were found at a deeper bathymetric range of 230 – 266 m. MRC and PISCES also differs regarding current and nutrient circulation processes.

At MRC, the presence of two distinct mechanisms controlling food supply has previously

Commented [U18]: A table showing the data used for the abiotic factors would be useful – the reader has no idea what the range of values are for each of these abiotic factors.

Commented [U19]: At these shallow depths, there is no depth effect per se – its just a proxy for temperature.

Commented [U20]: repeat

Commented [U21R20]: Repeats above

Commented [U22]: And how would these factors explain what you find? What else could you measure that might show positive responses?

been demonstrated (Davies *et al.* 2009; Duineveld *et al.* 2012; Findlay *et al.* 2013). Colonies at MRC receives warmer plankton-rich water thanks to a tidal down-welling of surface water which further circulates to colonies located at BR. Colonies at MR benefit from re-suspended matter from advected deep bottom water (Davies *et al.* 2009; Duineveld *et al.* 2012; Findlay *et al.* 2013).

The oceanographic and food supply regimes at the PISCES site are not yet as well characterised. Preliminary values from Particulate Organic Carbon (POC) data available from the JC073 Cruise (Roberts unpubl. data) revealed fourfold higher POC values for the MRC (MR: 42.70 ± 12.72µg L^{-1}; BR: 20.00 ± 3.18µg L^{-1}) than for the PISCES area (10.54 ± 5.35 µg L^{-1}). High POC values have been demonstrated to be an important carbon source for benthic communities including suspension feeding corals and gorgonians (Ribes *et al.* 1998; Houlbrèque and Ferrier-Pages 2009; Wagner *et al.* 2011). Moreover, Findlay *et al.* (2014) assessed the variability of nutrient and carbonate system dynamics at several Atlantic sites, including MRC and PISCES; MRC showed distinct temperature and salinity properties compared to PISCES (Fig. 5), as MRC has a marked coastal influence, and addition of fresh water run-off.

Oxygen concentration and Dissolved Inorganic Carbon (DIC) ~~were also analysed and~~ both ~~metrics~~ displayed lower values at PISCES than at MRC as a consequence of the deeper depth range of PISCES. The higher POC concentrations detected in MRC compared to PISCES, could also help to explain the higher number of well-developed cauliflower colonies in MRC, displaying a population with colonies that have different development degree (different sizes), compared to PISCES which display more homogeneous sizes and in general large individuals, which might indicate a more senescent population. Recent work has shown that the live portions of the coral reef mounds at Mingulay grow preferentially into the prevailing residual current direction (De Clippele *et al.* 2017) where the reef structure very probably slows flow

Commented [U23]: So…how does this information link to your findings?

Commented [U24]: This needs clarification. The sentence brings up nutrients and carbonates, then talks about temperature and salinity. Unclear what point is being made.

Commented [U25R24]:

Commented [U26]: Explain this argument more clearly. Why does high POC explain more cauliflower shaped colonies?

velocities to optimal levels for coral feeding (Orejas et al. 2016).

Colony size, layer thickness estimation and environmental factors

Total colony size and dead layer lengths measured here displayed a higher inter-colony variability than living layer lengths and LL : WC ratio. However, no significant differences could be detected between MRC and PISCES sites. Interestingly living layer length was positively correlated to the whole colony size whereas the LL : WC ratio was negatively correlated to the whole colony size (Fig. 4). This could suggest that as a colony develops and its growth rate slows down (as suggest by Brooke and Young 2009 and Lartaud *et al.* 2013 by comparing young and old polyp growth rates), the proportion of living polyps in the colony decreases. To our knowledge this constitutes the first quantitative analysis of the layering displayed by a reef framework forming CWC. The pattern here observed is similar to those in the facultative zooxanthellate scleractinian coral *Oculina varicosa* (Reed 2002). In Reed's work it was argued that the death of deep-dwelling azooxanthellate coral tissue was due to limited water flow in the core of these colonies because of the dense branching network (Reed 2002). Flow intensity has been previously demonstrated to influence capture rate of tropical corals (McFadden 1986; Helmuth and Sebens 1993; Johnson and Sebens 1993; Helmuth *et al.* 1997; Sebens *et al.* 1998; Hoogenboom *et al.* 2008). In CWC and for *L. pertusa* in particular, water velocities also impact capture efficiency (Purser *et al.* 2010; Orejas *et al.* 2016). However, no significant correlation between abiotic factors including average current speed and layer sizes could here be found to explain the reasons for the differences detected in the colonies at MRC. The 3D hydrodynamic model used to extract abiotic data (Moreno-Navas *et al.* 2014) has a 100 m spatial resolution, which is most probably too coarse to reveal the influence of ~~impact of the~~ hydrodynamics on development of~~n~~ a single colony~~y development~~.

It is important to take into account that the LL : WC ratio as calculated here, never

Commented [U27]: This seems illogical. The corals are facing directly into the current.

Commented [U28]: Thickness is probably better than length. If the authors agree, then this needs to be changed throughout the document.

Commented [U29]: MRC was also a lot more variable than PISCES, although that could be sampling artefact. Can you explain why that might be? .

exceeded 0.27 meaning that the living layer never represents more than one quarter of the whole colony size. Initial short-term laboratory experiments show that *L. pertusa* seemsed resilient to changes in water chemistry and lower pH conditions (Maier *et al.* 2013, Hennige *et al.* 2014a). Furthermore, reef building CWC such as *L. pertusa* have been foundd already to live very close to the aragonite saturation horizon (ASH) (Lunden *et al.* 2013). However, a recent study published by Hennige *et al.* (2015) shows that even though *L. pertusa* is able to physiologically adapt to OA conditions over a longer time period, its skeleton becomes significantly weaker, leading to breakage of the framework and higher susceptibility to bioerosion and mechanical damage. As the dead layer constitutes the great majority of a coral colony as shown by our measurements, dissolution of the underlying coral framework as thea consequences of OA, could undermine the structure of on CWC reefs and the habitats they support, could be worse than expected. A negative influence of low pH levels on the dead layer would lead to a weakening of the skeleton and its breakage, with the consequent destruction of the three-dimensional structure of the colonies and the reef.

Moreover, Tthe colonies analysed here have all been present for over a decade in the environment. The degradation of these long-lasting slow-growing colonies as a result of OA will have strong impact on the benthic community associated with CWC. Further investigation of colony layering and morphotypes at other reefs, as well as growth rate *in situ* validations, is needed to further investigate the use of LL : WC ratio measurements as a bio-indicator of colony health and susceptibility to OA.

Such colony health indicators are vital in order to develop robust protocols to monitor the change in CWC colony health and change over time, particularly to assess the effectiveness of deep-water Marine Protected Areas created for their long-term conservation. Recent work has shown the slow growth rate and low recovery potential of deep-water coral habitats at the

Commented [U30]: It is not clear what the point is here. How does this information relate to the findings of the study?

Commented [U31]: I don't think it is unexpected that the exposed framework is vulnerable to increased dissolution rates.

Commented [U32]: Suggest deleting or expanding this statement, its rather a non sequitur

Commented [U33]: Repeats above

Commented [U34]: Do you mean because of the amount of dead coral?

Darwin Mounds at 1,000 m water depth in the Rockall Trough (Huvenne et al. 2016). Our work demonstrates a simple metric that could be scaled up using autonomous survey approaches (see Wynn et al. 2014) to characterise large habitat areas and gather large datasets from many more coral colonies than possible in the present study. When combined with machine-learning image analysis and a robust understanding of deep-water MPA network connectivity (Fox et al. 2016) these approaches will revolutionise our understanding of deep-sea habitats and our ability to monitor them over time.

Commented [U35]: Similar methods could be applied to existing data from ROV surveys in different regions, creating a standardized measurement to compare live/dead ratios across regions.

Commented [U36]: I think this is rather overstating. This is a different method for measuring coral live:dead ratios, but I wouldn't say its revolutionary.

ACKNOWLEDGEMENTS

JV, JMN and JMR acknowledge additional support from University of Edinburgh Changing Ocean group. JV and CO acknowledge support from the IEO. We thank the captain, crew and scientific participants of RRS *James Cook* cruise 073 for assistance at sea.

FUNDING STATEMENT

This paper is a contribution to the UK Ocean Acidification Research Programme (NE/H017305/1) to JMR; funded by the Natural Environment Research Council, the Department for Energy and Climate Change, and the Department for Environment, Food and Rural Affairs) and the ATLAS project funded by the European Commission's H2020 scheme through Grant Agreement 678760. JV acknowledges additional support from a Natural Environment Research Council and British Geological Survey University Funding Initiative PhD stipend through the Centre for Doctoral Training in Oil & Gas.

REFERENCES

Arnaud-Haond S., van den Beld I.M.J., Becheler R., Orejas C., Menot L., Frank N., Grehan A., Bourillet J.F. (2015) Two "pillars" of cold-water corals reefs along Atlantic

European Margins: Prevalent association of *Madrepora occulata* with *Lophelia pertusa*, from reef to colony scale. *Deep-Sea Research Part II*, 1-10.

Barnes D.J. (1973) Growth in colonial scleractinians. *Bulletin of Marine Science* 23, 280-298.

Brooke S. and Young C.M. (2009) *In-situ* measurement of survival and growth of *Lophelia pertusa* in the northern Gulf of Mexico. *Marine Ecology Progress Series* 397,153-161.

Davies A.J., Duineveld G.C.A., Lavaleye M.S.S., Bergman M.J.N., van Haren H. and Roberts J.M. (2009) Downwelling and deep-water bottom currents as food supply mechnisms to the cold-water coral *Lophelia pertusa* (Scleractinia) at the Mingulay Reef complex. *Limnology and Oceanography* 54, 620-629.

De Clippele L., Gafeira J., Robert K., Hennige S., Lavaleye M., Duineveld G.C.A., Huvenne V.A.I. and Roberts J.M. (2017) Using novel acoustic and visual mapping tools to predict the small-scale spatial distribution of live biogenic reef framework in cold-water coral habitats. *Coral Reefs* 36, 255–268

De Mol L., Van Rooij D., Pirlet H., Greinert J., Frank N., Quemmerais F. and Henriet J.P. (2011) Cold-water coral habitats in the Penmarc'h and Guilvinec Canyons (Bay of Biscay): Deep-water versus shallow-water settings. *Marine Geology* 282, 40-52.

Dodds L.A., Roberts J.M., Taylor A.C. and Marubini F. (2007) Metabolic tolerance of the cold-water coral *Lopehlia pertusa* (Scleractinia) to temperature and dissolved oxygen change. *Journal of Experimental Biology and Ecology* 349, 205-214.

Dons C. (1944) Norges korallrev. *Kongelige Norske videnskabernes selskab forhandlinger* 16, 37-82.

Duineveld G.C.A., Jeffreys R.M., Lavaleye M.S.S., Davies A.J., Bergman M.J.N., Watmough T. and Witbaard R. (2012) Spatial and tidal variation in food supply to shallow cold-water coral reefs of the Mingulay Reef complex (Outer Hebrides, Scotland).

406 *Marine Ecology Progress Series* 444, 97-115.
407 **Dullo W.C.** (2005) Coral growth and reef growth: a brief review. *Facies* 51, 33-48.
408 **Duncan PM** (1877) On the rapidity of growth and variability of some Madreporaria on an
409 Atlantic cable, with remarks upon the rate of accumulation of foraminiferal deposits.
410 Proceedings of the Royal Society of London 26: 133-137
411 **Findlay H.S., Artioli Y., Moreno Navas J., Hennige S.J., Wicks L.C., Huvenne V.A.,**
412 **Woodward E.M. and Roberts J.M.** (2013) Tidal downwelling and implications for the
413 carbon biogeochemistry of cold-water corals in relation to future ocean acidification and
414 warming. *Global Change Biology* 19, 2708-2719.
415 **Findlay H.S., Hennige S.J., Wicks L.C., Moreno-Navas J., Malcom E., Woodward S. and**
416 **Roberts J.M.** (2014) Fine-scale nutrient and carbonate system dynamic around the cold-
417 water coral reefs in the Northeast Atlantic. *Scientific Reports* 4, 1-10.
418 **Form A.U. and Riebesell U.** (2011) Acclimation to ocean acidification during long-term CO_2
419 exposure in the cold-water coral *Lophelia pertusa*. *Global Change Biology* 18, 843-853.
420 **Foster A.B.** (1979) Phenotypic plasticity in the reef corals *Montastrea annularis* and
421 *Siderastrea sideria*. *Journal of Experimental Marine Biology and Ecology* 39, 25–54.
422 **Fox A., Henry L.A., Corne D.W. and Roberts J.M.** (2016) Sensitivity of a marine protected
423 area network to shifts in atmospheric state and ocean circulation. *Royal Society Open*
424 *Science* 3,160494
425 **Freiwald A., Wilson J.B. and Henrich R.** (1999) Grounding Pleistocene icebergs shape
426 recent deep-water coral reefs. *Sedimentary Geology* 125, 1-8.
427 **Freiwald A.** (2002) Reef-Forming Cold-Water Corals. In *Ocean Margin Systems*. Berlin
428 Heidelberg.
429 **Gass S.E. and Roberts J.M.** (2006) The occurrence of the cold-water coral *Lophelia pertusa*
430 (Scleractinia) on oil and gas platforms in the North Sea: Colony growth, recruitment and

environmental controls on distribution. *Marine Pollution Bulletin* 52, 549-559.

Gass S.E. and Roberts J.M. (2011) Growth and branching patterns of *Lophelia pertusa* (Scleractinia) from the North Sea. *Journal of the Marine Biological Association of the United Kingdom* 91, 831-835.

Gori A., Orejas C., Madurell T., Bramanti L., Martins M., Quintanilla E., Marti-Puig P., Lo Iacono C., Puis P., Requena S., Greenacre M. and Gili JM. (2013) Bathymetrical distribution and size structure of cold-water coral populations in the Cap de Creus and Lacaze-Duthiers canyons (northwestern Mediterranean). *Biogeosciences* 10, 2049-2060.

Grigg R.W. (1972) Orientation and growth form of sea fans. *Limnology and Oceanography* 17:185-192.

Hall-Spencer J.M., Rodolfo-Metalpa R., Martin S., Ransome E., Fine M., Turner S.M., Rowley S.J., Tedesco D. and Buia M.C. (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* 454, 96-99.

Helmuth B.S.T. and Sebens K.P. (1993) The influence of colony morphology and orientation to flow on particle capture by the scleractinian coral *Agaricia agaricites* (Linnaeus). *Journal of Experimental Marine Ecology* 165, 251-278.

Helmuth B.S.T., Sebens K.P. and Daniel T.L. (1997) Morphological variation in coral aggregations: branch spacing and mass flux to coral tissues. *Journal of Experimental Marine Biology and Ecology* 209: 233-259.

Hennige S.J., Wicks L.C., Kamenos N.A., Bakker D., Findlay H.S., Dumousseaud C. and Roberts J.M. (2014a) Short-term metabolic and growth responses of the cold-water coral *Lophelia pertusa* to ocean acidification. *Deep-Sea Research Part II* 99, 27-35.

Hennige S.J., Morrison C.L., Form A.U., Büscher J., Kamenos N.A. and Roberts J.M. (2014b) Self-recognition in coral facilitates deep-sea habitat engineering. *Scientific*

Reports 4, 1-7.

Hennige S.J., Wicks L.C., Kamenos N.A., Perna G., Findlay H.S. and Roberts J.M. (2015) Hidden impacts of ocean acidification to live and dead coral framework. *Proceedings of the Royal Society B* 282: 1-10.

Henry L.A., Davies A.J. and Roberts J.M. (2010) Beta diversity of cold-water coral reef communities off western Scotland. *Coral Reefs* 29, 427-436.

Hoogenboom M.O., Connolly S.R. and Anthony K.R.N. (2008) Interactions between morphological and physiological plasticity optimize energy acquisition in corals. *Ecology* 89, 1144-1154.

Houlbrèque F. and Ferrier-Pages C. (2009) Heterotrophy in tropical scleractinian corals. *Biological Reviews* 84, 1-17.

Hughes T.P. (1987) Skeletal density and growth form of corals. *Marine Ecology Progress Series* 35, 259-266.

Johnson A.S. and Sebens K.P. (1993) Consequences of a flattened morphology: effects of flow on feeding rates of scleractinian coral *Meandrina meandrites*. *Marine Ecology Progress Series* 99, 99-114.

Larcom E.A., Mckean D.L., Brooks J.M. and Fisher C.R. (2014) Growth rates densities and distribution of *Lophelia pertusa* on artificial structures in the Gulf of Mexico. *Deep-sea Research* 15, 101-109.

Lartaud F., Pareige S., De Rafelis M., Feuillassier L., Bideau M., Peru E., Romans P., Alcala F. and Le Bris N. (2013) A new approach for assessing cold-water coral growth *in situ* using fluoresdent calcein staining. *Aquatic Living Resources* 26, 187-196.

Lunden J.J., Georgian S.E. and Cordes E.E. (2013) Aragonite saturation states at cold-water coral reefs structured by *Lophelia pertusa* in the nothern Gulf of Mexico. *Limnology and Oceanography* 58, 354-362.

Maier C., Schubert A., Sanchez M.M.B., Weinbauer M.G., Watremez P. and Gattuso J.P. (2013) End of century pCO_2 levels do not impact calcification in Mediterranean cold-water corals. *PLoS ONE* 8, 1-9.

McCulloch M., Trotter J., Montagna P. Falter J., Dunbar R., Freiwlad A., Försterra G., López Correa M., Maier C., Rüggeberg A and Taviani M. (2012) Resilience of cold-water scleractinian corals to ocean acidification: Boron isotopic systematics of pH and saturation state up-regulation. *Geochimica et Cosmochimica* 87, 21-34.

McFadden C.S. (1986) Colony fission increases particle capture rates of a soft coral: advantages of being a small colony. *Journal of Experimental Marine Biology and Ecology* 103, 1-20.

Mienis F., de Stigter H.C., White M., Duineveld G., de Haas H., van Weering T.C.E. (2007) Hydrodynamic controls on cold-water coral growth and carbonate-mound development at the SW and SE Rockall Trough Margin, NE Atlantic Ocean. *Deep-Sea Research I* 54, 1641-1654.

Miller M.W. (1995) Growth of a temperate coral: effects of temperature, light, depth and heterotrophy. *Marine Ecology Progress Series* 122, 217-225.

Moreno-Navas J., Miller P.L., Henry L.A., Hennige S.J. and Roberts J.M. (2014) Ecohydrodynamics of cold-water coral reefs: a case study of the Mingulay Reef Complex (western Scotland). *PloS One* 9, 1-12.

Movilla J., Orejas C., Calvo E., Gori A., López-Sanz À., Grinyó J., Domínguez-Carrió C. and Pelejero C. (2014) Differential response of two Mediterranean cold-water coral species to ocean acidification. Coral Reefs 33, 675-686.

Naumann M.S., Orejas C. and Ferrier-Pagès C. (2014) Species-specific physiological response by the cold-water corals *Lophelia pertusa* and *Madrepora oculata* to variations within their natural temperature range. Deep-Sea Research II 99, 36-41.

506 **Olariaga A., Gori A., Orejas C., Gili J.M. and Roberts J.M.** (2009) Development of an
507 autonomous aquarium system for maintaining deep corals. *Oceanography* 22, 44-45.
508 **Orejas C., Gori A. and Gili J. M.** (2008) Growth rates of live *Lophelia pertusa* and
509 *Madrepora oculata* from Mediterranean Sea maintained in aquaria. *Coral Reefs* 27, 255-
510 255.
511 **Orejas C., Gori A., Lo Iacono C., Puig P., Gili J.M. and Dale M.R.T.** (2009) Cold-water
512 corals in the Cap de Creus canyon, northwestern Mediterranean: spatial distribution,
513 density and anthropogenic impact. *Marine Ecology Progress Series* 397, 37-51.
514 **Orejas C., Ferrier-Pages C., Reynaud S., Tsounis G., Allemand D. and Gili J.M.** (2011a)
515 Experimental comparison of skeletal growth rates in the cold-water coral *Madrepora*
516 *oculata* Linnaeus, 1758 and three tropical scleractinian corals. *Journal of Experimental*
517 *Marine Biology and Ecology* 405, 1-5.
518 **Orejas C., Ferrier-Pages C., Reynaud S., Tsounis G., Allemand D. and Gili J.M.** (2011b)
519 Long-term growth rates of four Mediterranean cold-water coral species maintained in
520 aquaria. *Marine Ecology Progress Series* 429, 57-65.
521 **Orejas C., Gori A., Rad-Menéndez C., Last K.S., Davies A.J., Beveridge C.M., Sadd D.,**
522 **Kiriakoulakis K., Witte U. and Roberts J.M.** (2016) Capture efficiency and feeding
523 behaviour of the cold-water coral *Lophelia pertusa*: flow speed and food size make a
524 difference. *Journal of Experimental Marine Biology and Ecology*.
525 **Purser A., Larsson A.I., Thomsen L. and van Oevelen D.** (2010) The influence of flow
526 velocity and food concentation on *Lophelia pertusa* zooplankton capture rates. *Journal of*
527 *Experimental Marine Biology and Ecology* 395, 55-62.
528 **Purser A., Orejas C., Gori A., Tong R., Unnithan V. and Thomsen L.** (2013) Local
529 variation in the distribution of benthic megafauna species associated with cold-water
530 coral reefs on the Norwegian margin. *Continental Shelf Research* 54, 37-51.

Reed J.K. (2002) Deep-water Oculina coral reefs of Florida: biology, impacts and management. *Hydrobiologia* 471, 43-55.

Ribes M., Coma R. and Gili J.M. (1998) Heterotrophic feeding by gorgonian corals with symbiotic zooxanthella. *Limnology and Oceanography* 43, 1170-1179.

Roberts J.M. and Anderson R.M. (2002) A new laboratory method for monitoring deep-sea coral polyp behaviour. *Hydrobiologia* 471, 143-148.

Roberts J.M., Brown C.J., Long D. and Bates C.R. (2005) Acoustic mapping using a multibeam echosounder reveals cold-water coral reefs and surrounding habitats. *Coral Reefs* 24, 654-669.

Roberts J.M., Wheeler A.J. and Freiwald A. (2006) Reefs of the deep: the biology and geology of cold-water coral ecosystems. *Science* 312, 543-547.

Roberts J.M., Davies A.J., Henry L.A., Dodds L.A., Duineveld G.C.A., Lavaleye M.S.S., Maier C., van Soest R.W.M., Bergman M.J.N., Hühnerbach V., Huvenne V.A.I., Sinclair D.J., Watmough T., Long D., Green S.L. and van Haren H. (2009) Mingulay reef complex: an interdisciplinary study of cold-water coral habitat, hydrography and biodiversity. *Marine Ecology Progress Series* 397, 139-151.

Roberts J.M. and shipboard party (2013) Changing Oceans Expedition 2012. *RRS James Cook 073 Cruise Report.* Heriot-Watt University, 224 pp.

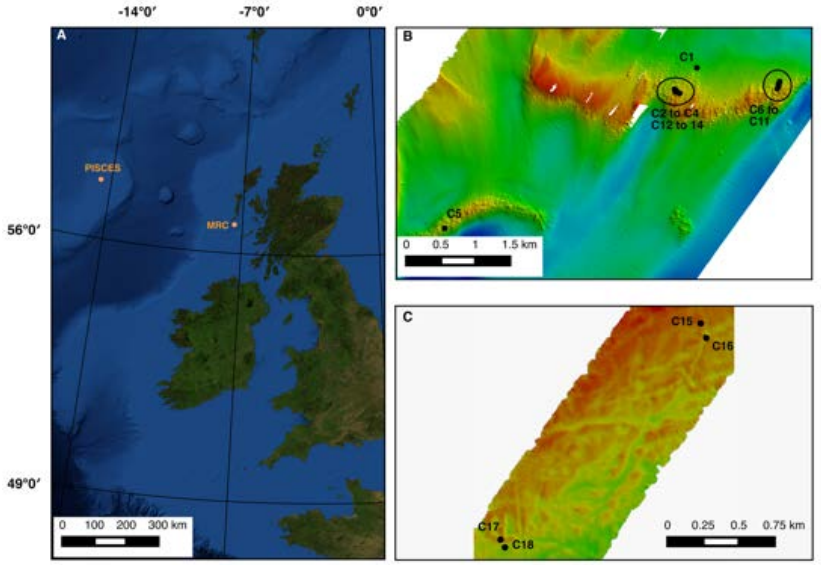
Rogers A. (2004) The biology, ecology and vulnerability of deep-water coral reefs. *IUCN*, 13pp.

Sebens K.P., Grace S.P., Helmuth B.S.T., Maney E.J. and Miles J.S. (1998) Water flow and prey capture by three scleractinian corals *Madracis mirabilis, Monstastrea cavernosa* and *Porites porites*, in a field enclosure. *Marine Biology* 131, 347-360.

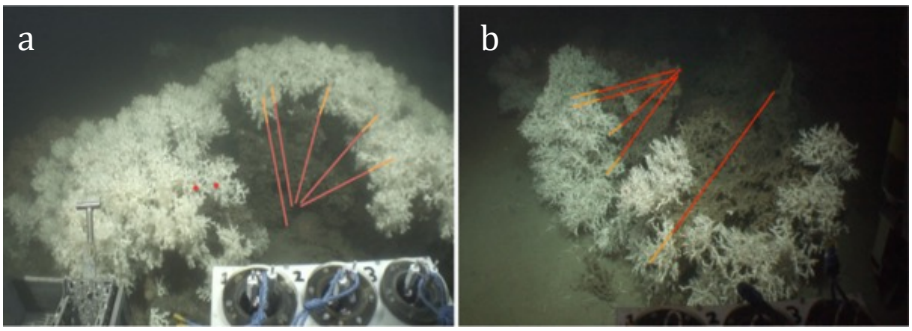
554 **Smith L.W., Barshis D. and Birkeland C.** (2007) Phenotypic plasticity for skeletal growth,
555 density and calcyfication of *Porites lobata* in response to habitat type. *Corel Reefs* 26,
556 559-567.
557 **Thresher R.E.** (2009) Environmental and compositional correlates of growth rate in deep-
558 water bamboo corals (Gorgonacea; Isididae). *Marine Ecology Progress Series* 397, 187-
559 196.
560 **Via S. and Lande R.** (1985) Genotype-environment interaction and the evolution of
561 phenotypic plasticity. *Evolution* 39, 505–522.
562 **Wagner H., Purser A., Thomsen L., Jesus C.C. and Lundälv T.** (2011) Particulate organic
563 matter fluxes and hydrodynamics at the Tisler cold-water coral reef. *Journal of Marine*
564 *Systems* 85:19-29.
565 **Willis B.L. and Ayre D.J.** (1985) Asexual reproduction and genetic determination of growth
566 form in the coral *Pavona cactus*: Biochemical genetic and immunogenic evidence.
567 *Oecologia* 65, 516-525.
568 **Wilson J.B.** (1979) Patch Development of the Deep-Water Coral *Lophelia pertusa* on
569 Rockall Bank. *Journal of the Marine Biological Association of the United Kingdom* 59,
570 165-177.
571 **Wynn R.B., Huvenne V.A.I., Le Bas T.P., Murton B.J., Connelly D.P., Bett B.J., Ruhl**
572 **H.A., Morris K.J., Peakall J., Parsons D.R., Sumner E.J., Darby S.E., Dorrell R.M.**
573 **and Hunt J.E.** (2014). Autonomous underwater vehicles (AUVs): their past, presence
574 and future contributions to the advancement of marine geoscience. *Marine Geology* 352,
575 451–468.

Figures and Tables

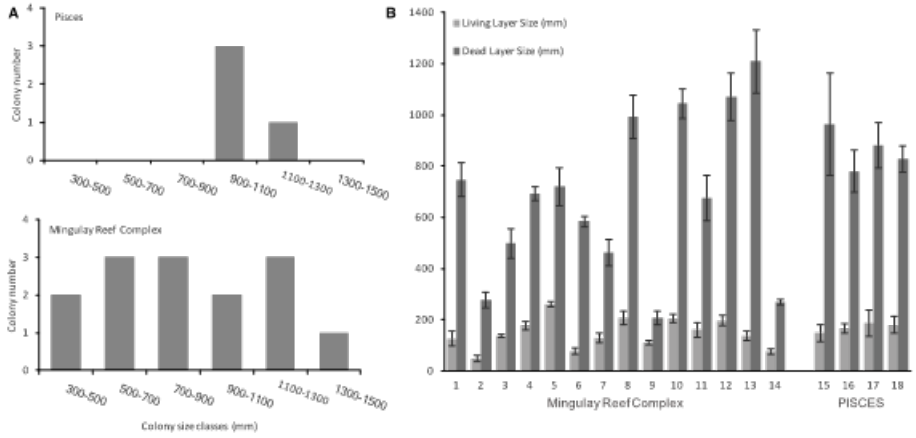
Fig. 1. (A) Mingulay Reef Complex (MRC) and PISCES area location offshore Scotland (B) Colonies 1 to 14 (C1 to C14) locations within MRC. Note that colony 5 (C5) is the only colony located on Banana Reef (C) Colonies 15 to 18 (C15 to C18) locations within PISCES area.



583 Fig. 2. *Lophelia pertusa* colonies from a) Mingulay Reef (MRC) and b) PISCES area. Images
584 show an example of the five colony measurements performed for each colony. Dead layer
585 thickness was measured along the red lines whereas living layer sizes was measured along the
586 orange lines. Laser beams (100 mm separation) are highlight by red dots on both photographs.
587 Image a) also shows the sampling box (400 mm length) used as scale in cases where the laser
588 beams were not clearly visible.



589 Fig. 3. *Lophelia pertusa* A) number of colonies belonging to the different size ranges detected
590 in the Mingulay Reef Complex (MRC) and the PISCES area and B) Living (grey) and dead
591 (antracit) layer sizes for the 20 *L. pertusa* colonies analysed (14 from MRC, including colony
592 5 which has been recorded in Banana Reef; 5 from PISCES). Error bars display the SD.



593

Figure 4: Scatterplot of living layer thickness and L: W ratio as a function of total colony sizes. Measurements from MRC colonies are displayed in black; measurements from PISCES colonies are displayed in grey. Regression lines for each plot are traced in green.

Commented [U37]: Add R-square values

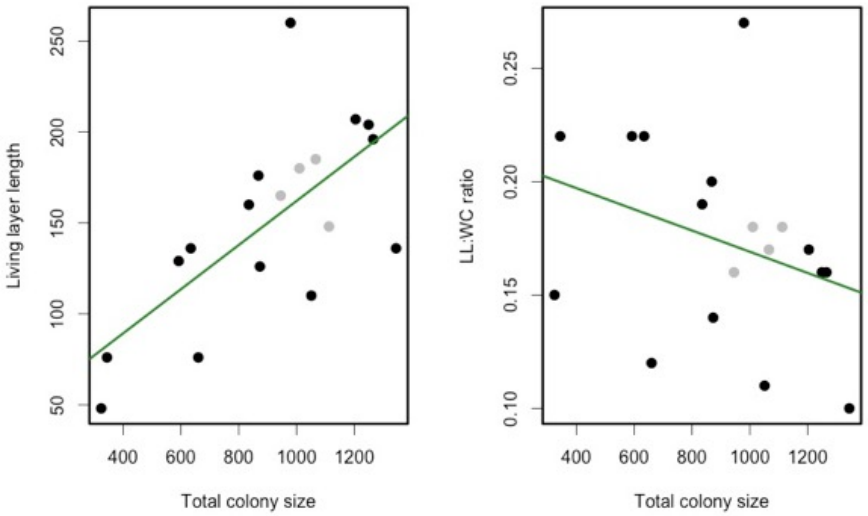
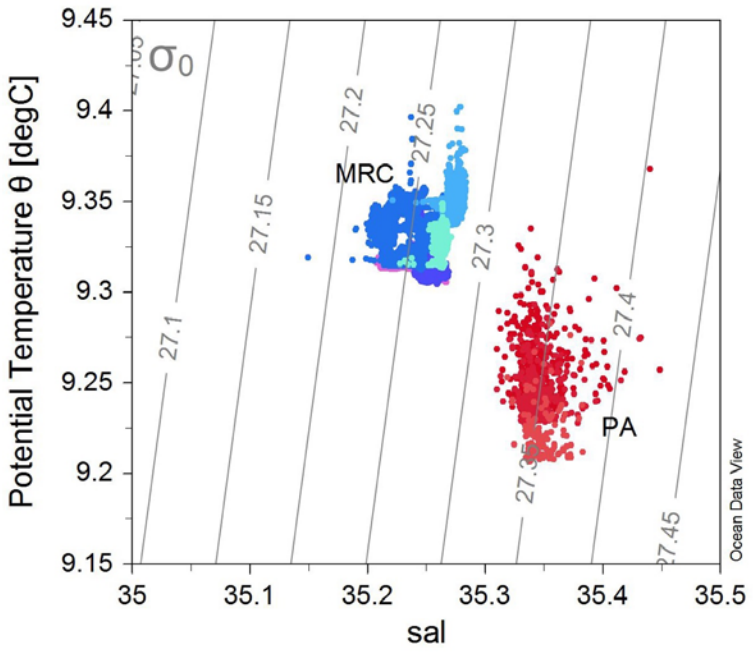


Figure 5: T-S plot showing the potential temperature *vs* salinity (sal), with isopycnals (grey lines) for all the ROV transects. Colours represent the different transects (and sites): blue colours show the transects within the Mingulay Reef Complex (MRC) while red colours show the transects within the PISCES areas (PA).



Commented [U38]: But you don't mention temperature as an abiotic factor, which would make sense given the influence of temperature on growth..

Table 1: Transect number, location, coordinates (latitude longitude) at the start and the end of each transect, depth (m) at the start and the end of the transect, length (m) and number of selected colonies analyzed in this study. MR: Mingulay Reef, BR: Banana Reef.

Commented [U39]: This is the US spelling, but the rest of the manuscript uses UK spelling. Needs consistency.

Transect	Location	Transect coordinates				Transect length (m)	Start depth (m)	End depth (m)	Number of selected colonies
		From		To					
1	MR	56°N 49.61	7°W 23.39	56°N 49.52	7°W 23.48	250	157	179	1
3		56°N 49.38	7°W 23.71	56°N 57.59	7°W 13.04	63	133	127	2
5		56°N 82.27	7°W 39.51	56°N 82.29	7°W 39.48	152	133	130	1
8		56°N 49.59	7°W 22.21	56°N 49.29	7°W 22.85	1236	167	130	6
10		56°N 49.36	7°W 23.69	56°N 49.38	7°W 23.68	84	130	127	2
41		56°N 49.55	7°W 23.49	56°N 49.43	7°W 23.3	238	150	127	1
7	BR	56°N 48.13	7°W 27.01	56°N 48.39	7°W 25.98	1226	145	155	1
31	PISCES	57°N 61.01	14°W 49.25	57°N 60.58	14°W 49.67	1865	262	260	2
32		57°N 59.49	14°W 51.27	57°N 59.49	14°W 50.96	613	262	261	2
									(Total = 18)

605 **Table 2**: *Lophelia pertusa* colony layer and whole size measurements (mm ± SD) and ~~L~~living
606 layer: Whole size ratio estimation (LL: WC) and age estimation based on Gass and Roberts
607 2006. * Colony 5 is the only colony present at Banana Reef. Transect number is displayed
608 between brackets in the second column.

Area	Colony number	Dead layer (mm)	Living layer (mm)	Total colony size (mm)	Living layer : whole size ratio	Age estimation (years)
Mingulay Reef Complex	1 (1)	747 ± 66	126 ± 30	873 ± 90	0.14 ± 0.02	34.9 ± 7.9
	2 (3)	276 ± 32	48 ± 13	324 ± 43	0.15 ± 0.02	12.9 ± 3.1
	3 (3)	498 ± 57	136 ± 7	634 ± 64	0.22 ± 0.01	25.3 ± 5.7
	4 (5)	692 ±25	176 ± 16	868 ± 32	0.20 ± 0.02	34.7 ± 7.1
	5* (7)	719 ± 75	260 ± 10	979 ± 75	0.27 ± 0.02	39.1 ± 8.4
	6 (8)	584 ± 21	76 ± 13	660 ± 23	0.12 ± 0.02	26.4 ± 5.4
	7 (8)	463 ± 52	129 ± 19	592 ± 37	0.22 ± 0.05	23.7 ± 5.0
	8 (8)	997 ± 73	207 ± 26	1204 ± 87	0.17 ± 0.02	48.1 ± 10.3
	9 (8)	940 ± 78	110 ± 7	1051 ± 79	0.11 ± 0.01	42.0 ± 9.0
	10 (8)	1045 ± 58	204 ± 17	1249 ± 66	0.16 ± 0.01	49.9 ± 10.4
	11 (8)	675 ± 89	160 ± 28	835 ± 104	0.19 ± 0.03	33.3 ± 7.8
	12 (10)	1070 ± 92	196 ± 20	1265 ± 95	0.16 ± 0.02	50.5 ± 10.9
	13 (10)	1208 ± 124	136 ± 18	1344 ± 115	0.10 ± 0.02	53.7 ± 11.7
	14 (41)	268 ± 9	76 ± 10	344 ± 19	0.22 ±0.02	13.7 ± 2.9
Pisces	15 (31)	964 ± 200	148 ± 33	1112 ± 226	0.13 ± 0.02	44.4 ± 12.5
	16 (31)	780 ± 82	165 ± 19	945 ± 70	0.18 ± 0.03	37.7 ± 8.1
	17 (32)	946 ± 181	181 ± 38	1057 ± 155	0.17 ± 0.03	45.0 ± 10.9
	18 (32)	828 ± 53	180 ± 32	1010 ± 71	0.18 ± 0.03	40.3 ± 8.6

609

Commented [U40]: There are 4 colonies here but only 3 mentioned elsewhere.

610 **Table 3**: Two sided two samples Wilcoxon test comparing mean dead layer thickness, mean
611 living layer thickness, mean whole colony size and mean LL:WC ratio between MRC and
612 Pisces sites.

	Two-sample Wilcoxon test	
	Wilcoxon W	p-Value
Dead layer thickness	14	**0.1515**
Living layer thickness	15	**0.1837**
Whole colony size	19	**0.3817**
LL:WC ratio	29.5	**0.915**

613