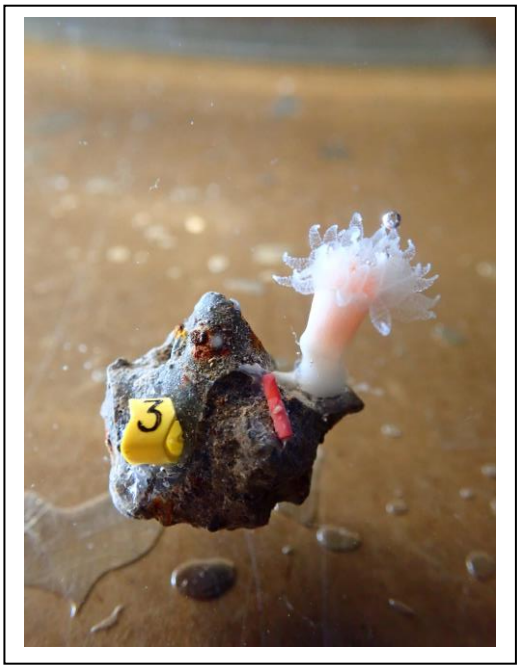
UNIVERSITY OF GOTHENBURG

DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

RESTORATION OF *DESMOPHYLLUM PERTUSUM: HOW POLYP GROWTH AND SURVIVAL IS AFFECTED BY SUBSTRATE AND NUTRITION, A 3D-MODEL ANALYSIS*



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Cover illustration: Whole fragment of a *Desmophyllum pertusum* polyp attached to a piece of metallurgic slag, taken by the author.

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Abstract

Desmophyllum pertusum is a cold-water coral found all over the world, including Sweden. It has a large impact on the ecology in deeper water with a similar amount of biodiversity as tropical coral reefs. During the recent decades almost all the reefs within Swedish waters have been destroyed, mostly due to bottom trawling. So far, the establishment of MPAs and a national park has helped prevent further destruction, but it has been concluded that active restoration is also necessary for the species' recovery, with microfragmentation emerging as a viable solution.

The primary aim of this study was to observe if the growth speed of fragmented polyps were affected by either substrate they were attached to, or colony they originated from. The secondary aim was to observe if the growth speed and survival of split coral fragments were affected by nutrition availability. Growth was measured using photogrammetry where images were taken at the beginning and after 10.5 weeks of treatment and then rendered into 3D-models after. The mean and volumetric growth was calculated by comparing the models in CloudCompare and MeshLab and then analysed using RStudio.

Mean growth for all whole coral fragments measured between ~0.97-0.442 mm and the volumetric growth varied between ~7.51-30.6 %. The mean growth for the split polyps varied between ~0.122-0.462 mm with most tissue-face up fragments generating a new head. While growth was observed in all the corals analysed, the size of growth from the model comparisons was unrealistic and yielded no significant results for any of the statistical tests. This study was limited by several factors including low-quality models, no calcein staining, and time limitations. In conclusion this thesis should be seen as a pilot study and if the above issues were to be solved in a future study more accurate results may be obtained.

1. Introduction

1.1 Cold water corals and restoration

During recent decades ecosystem-building organisms in the deep sea have been decreasing rapidly due to anthropogenic exploitation, primarily from the fishing industries and deep-water mining (Davies et al., 2007). Just like with tropical reefs it is just as important to protect cold water coral (CWC) reefs and their biodiversity. Since it's believed that natural conservation strategies like marine protected areas (MPAs) aren't enough for successful coral restoration, active coral recovery efforts have become integral to mitigate losses (Lock et al., 2022). Even though *D. pertusum* and other species of CWC share the importance of keeping their ecosystems thriving with their tropical counterparts and research interest has dramatically increased since the 1990s (Roberts & Cairns, 2014), there is still less research knowledge about CWCs compared to tropical species (Comms, 2024).

Two of the largest problems with restoration of CWC and the deep sea in general are a lack of public awareness and the cost of restoration compared to coastal environments, likely by two to three orders of magnitude (Van Dover et al., 2014). The same author also argued that it is mostly due to the inaccessibility and technical requirements of the deep sea compared to restoration efforts in shallow water. Even though CWC are ecosystem builders and house many important commercial species, they are not often talked about or even known about in non-scientific circles, which might lead to difficulties in gaining monetary support for their recovery.

To combat the increasing destruction of marine environments, the European union (EU) created the nature restoration regulation which went into effect in August of 2024 across the entire EU. Since around 80% of the habitats in the union have been damaged in some way the goal with the regulation is to restore 20% of the EU's degraded ecosystems by 2030, and all ecosystems by 2050. By restoring CWC the hope is that it will also encourage the return of other marine species which will be crucial to reduce risks to food security and increase resilience of marine food systems (Regulation (EU) 2022/869, 2024). To aid with the restoration efforts the Kosterfjord-Väderöfjord area was turned into a Natura 2000 site of community interest (SCI) in 2005, a special area of conservation (SAC) in 2011, and the Koster sea was turned into a national park in 2009 (Länstyrelsen Västra Götalands Län, 2026; *Natura 2000 SDF - SE0520170*, 2025). This means that the areas that contain reefs are legally protected from among other things bottom trawling, marine littering, anchoring in proximity to the reefs, and construction works (Naturvårdsverket, 2024; *Om Projektet - LIFE Lophelia*, n.d.).

1.2 *Desmophyllum pertusum* and substrate preferences

The cold-water coral *Desmophyllum pertusum* is a reef-forming organism that exists all over the world in deeper oceans with temperatures between 4 and 12 °C. Their usual habitats lie between 80-3000 meters, but in some places in Norway they can be found as shallow as 39 meters (Rogers, 1999; Fosså et al., 2002). These creatures can construct enormous reefs over 35 km long and colonies that are estimated to be around 10,000 years old, while containing the same amount biodiversity as tropical reefs (Rogers, 1999; Jonsson et al., 2004; Fosså et al., 2005). Growth rates of *D. pertusum in situ* can reach up to 5.5 mm/year, which is slower compared to more massive shallow water coral species (4-25 mm/year), and much slower in relation to branching shallow

water coral species that can grow at speeds between 100-200 mm/year (Rogers, 1999). However, there is evidence that *D. pertusum* can grow up to 17 mm/year *ex situ* (Orejas et al., 2008) which shows evidence that restoration efforts of CWC reefs may be improved by not only letting larvae settle naturally on artificial reefs, but also growing fragments in nurseries and transplanting them onto substrates in the ocean. Coral fragmentation is a method of restoring coral reefs by taking small fragments of healthy coral and growing new colonies from these (NOAA, 2024). These cultivations have proven to be successful in active reef restoration and are now widely applied on thousands of coastal coral nurseries and over 100 tropical coral species (Rinkevich, 2019, 2021).

Research has also shown that substrates enriched with magnesium carbonate, magnesium sulphate, and strontium significantly increase tropical coral settlement (Yus et al., 2024). Additionally, previous experiments have already been done on substrate settling preference in the larval stage of *D. pertusum* showing a preference for metallurgic slag (A. Larsson, personal communication, April 21, 2026), and studies have also indicated that *ex situ*, the *D. pertusum* larvae were significantly more interested in ground granulated blast furnace slag (GGBFG) compared to normal Portland cement (PC) while some ceramics were on average better. It is also known that larvae settling can be enhanced with the addition of strontianite ($SrCO_3$) and magnesium to the ceramics (Strömberg et al., 2025). *In situ* field studies (Strömberg & Larsson, 2025) suggest that the complexity of a reef structure (using metallurgic slag to create different surfaces) has a larger effect on species richness than the material composition of the reef itself. Research by Maier (2008) has also shown that *D. pertusum* has great potential for regeneration after being split and that the growth direction can change after fragmentation. There have also been smaller studies on how fragment size and polyp number influence growth rate (Engström, 2025) and research on both diet and substrate settling variation in *D. pertusum*. This coral is indicated to be an opportunistic feeder and is able to efficiently incorporate different nutrients of various sizes into its diet from copepods to dissolved organic matter, which makes the species quite flexible in adapting to diverse environments and food availability (Mueller et al., 2014). In 2019 the LIFE *Lophelia* project was started at Tjärnö Marine Laboratory (TML) to develop methods of restoration for *D. pertusum* using artificial reef structures with the end goal of large-scale conservation of the reefs and fauna that inhabit the surrounding area (*Om Projektet - LIFE Lophelia*, n.d.). However, there have to my knowledge been few studies (or none) looking at how growth rate of whole, microfragmented *D. pertusum* polyps are affected by the substrate it is attached to or how split coral polyps are affected by nutrients in the water. The goal of this thesis is to contribute more knowledge to these fields using non-invasive methods (Lange & Perry, 2020) of measuring their growth.

1.3 Aim of the thesis

The primary aim of this study is to observe how different substrates (two types of ceramics, cement, and metallurgic slag) affects growth of single *D. pertusum* polyps from different colony origins. The secondary aim of this thesis is to check how the splitting of polyps and the subsequent regeneration will be affected by different amounts of concentrated amino acid solution and whether the polyp fragment is placed tissue up or down. Growth is measured using photogrammetry and the models from the initial and final measurements are compared in a separate program.

2. Materials and methods

2.1 Previous collection and keeping of *Desmophyllum pertusum*

Fragments of coral colonies were collected from the Tisler reef (58°59'7"N, 10°28'2"E) on the 21/11/2024 at a depth of 115-130 m using an ROV (Ocean Modules v8 m500). The samples were transported back to TML and kept in a flow-through aquarium within a dark thermal constant room with a maintained average temperature of 8°C both in the air and water, and a salinity between 32-34 PSU taken from around 45 meters depth. They have since been fed three times/week with Ocean Nutrition™ red plankton cube that was thawed out in the same kind of filtered saltwater as in the aquariums without turning off the waterflow.

2.2 Preparations

3.2.1 Substrate preparation

A total of five kinds of substrate were used for the entirety of the project. For the photogrammetry part of the project with whole polyps, the substrates consisted of ceramic plugs (CP), ceramic plugs enriched with strontium and magnesium (ECP), Portland cement (PC), and pieces of metallurgic slag (MS). For the split coral polyps, carbonated ceramic plugs (CCP) were the only substrate used. Once the substrates were prepared identification numbers and red plastic strips to be used as size reference for the photogrammetry modelling were attached using cyanoacrylate based coral glue (CBCG). The plastic strips were cut into 5 mm lengths using a razor blade under a stereo microscope on top of a ruler to be as accurate as possible.

2.2.2 Fragmentation

The corals were fragmented for the substrate experiment by hand into various sizes (⌀ 1.87–4.05 mm) containing one polyp each from the newer and smaller polyps from older colonies. The polyps were selected based on relative size to each other and were selected from five different colonies. A total of 20 polyps were fragmented for the four kinds of substrates, with five replicates per substrate. The 20 polyps were then placed haphazardly around in their aquarium (Fig 2B). Additional polyps were clipped from five colonies for the split polyp nutrition experiment using a coral cutter mini. The clipped polyps were then split into two or three pieces using a razor blade for a total of 24 replicates, where twelve were placed with polyp interior facing down and twelve were placed with polyp interior facing up. The split polyps' treatment and placement was randomized using ChatGPT 4.1 with the prompt "randomly assign 24 individuals into 4 blocks evenly". This resulted in each aquarium having three polyps with tissue face up and three polyps having tissue face down towards the substrate. All replicates for both experiments were glued onto the substrates close to the measuring strip using CBCG, taking care to use as little as possible so the fragment would still be in contact with the substrate as much as possible.

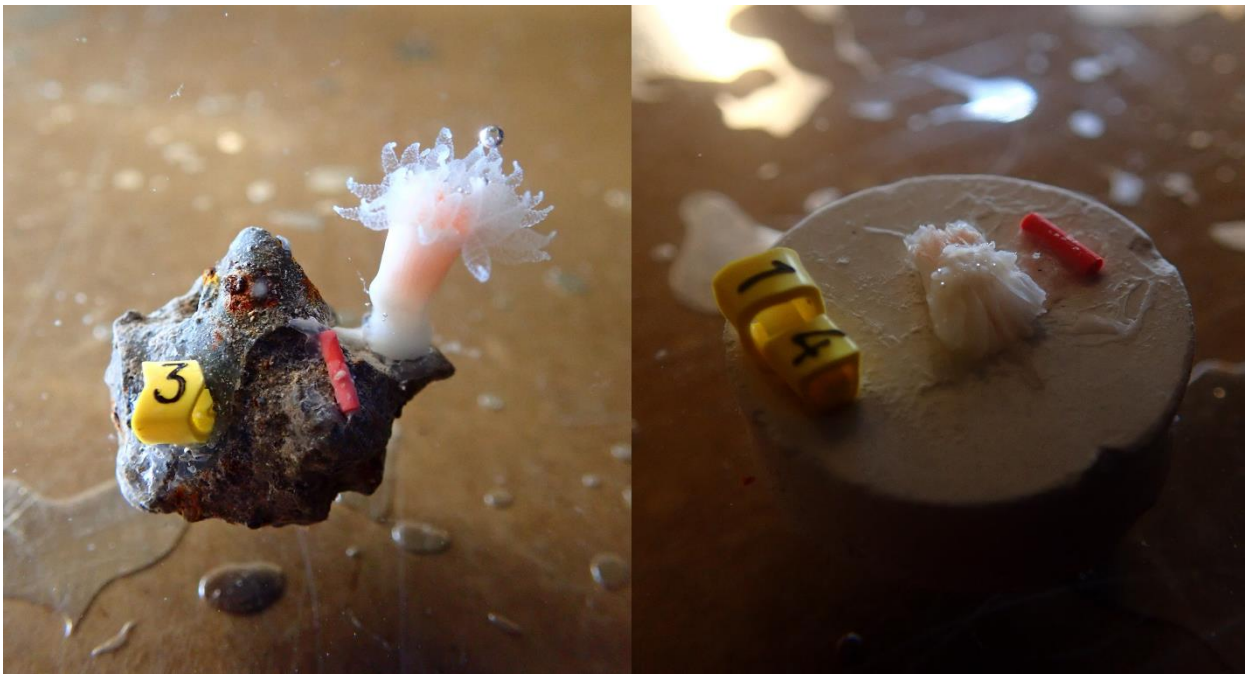

Figure 1: Examples on how coral polyps, ID-numbers, and reference strip were placed for whole polyps (left) and split polyps (right).

2.3 Habitats and feeding routines

2.3.1 Habitats

A total of five aquariums were used. For the substrate experiment a larger 33 l aquarium was used, while four smaller 12 l aquariums were used for the housing of the split polyps for the nutrition experiment (Fig 2A). All water was filtered through both a 50 µm filter and a 5 µm filter before entering the aquariums. There was a constant waterflow of ~0.45 l/min in the smaller tanks and ~2.5 l/min in the large tank. The polyps were kept in these conditions for 10.5 weeks in total.

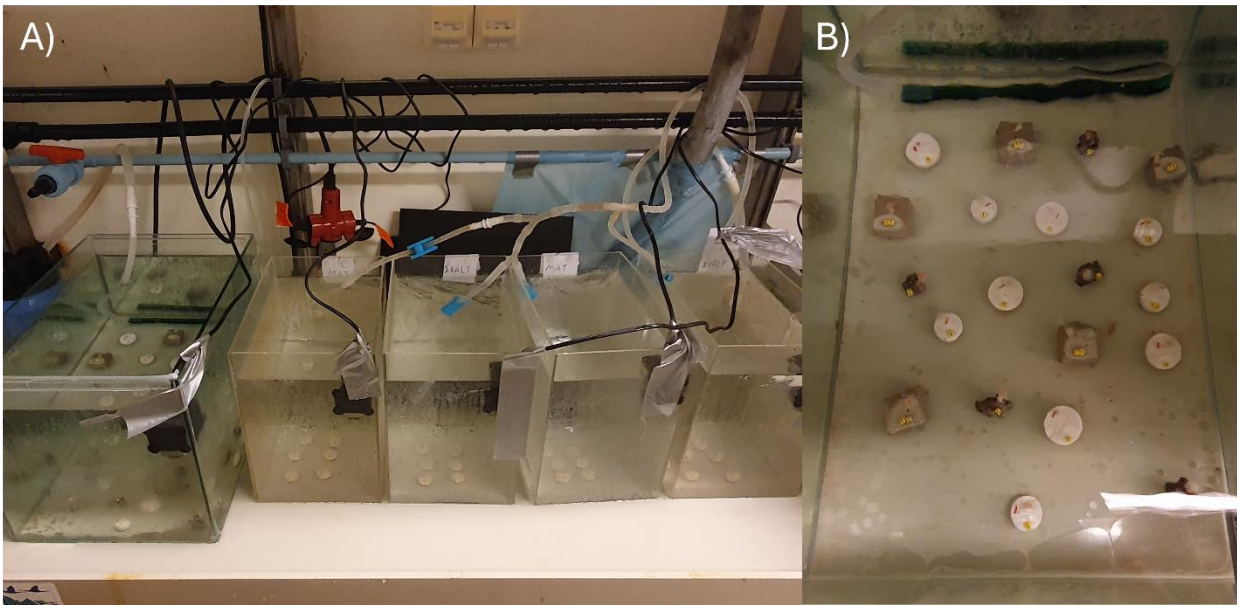

Figure 2 A) The habitats used for the substrate (left) and nutrition (right) experiments. B) The whole polyps sitting on different substrates and randomly placed in the aquarium.

2.3.2 Feeding routines

Nutrients and treatment varied among tanks. In the larger tank where the whole polyps on different substrates resided (Fig 2B), a regular feeding schedule was kept and they were fed three times a week with one 5g Ocean Nutrition™ red plankton cube that was thawed out in the same kind of filtered water as in the aquariums, and 1 ml Ocean Nutrition™ reef pulse coral nutrition powder. The waterflow was then turned off for 30 minutes to ensure that most of the food would be picked up by the corals instead of flowing out of the aquarium. For the split polyps the feeding was different. Since the focus was to monitor coral growth depending on dissolved nutrition, treatment 1 received 0.25 ml of Fauna Marin AMIN amino acid solution mixed into 25 ml filtered saltwater per aquarium everyday Monday through Friday while treatment 2 did not receive any food. Aquariums 3 and 4 were used as replicates and got the same amount of nutrition as treatment 1 and 2, respectively. The water for all aquariums was turned off for 3 hours during feeding to keep the nutrition solution longer in the treatment aquariums. This program was kept for the entirety of the project with an exception for April 3rd and April 6th due to Easter holidays. To give the whole polyps time to use up stored nutrients the last feeding was done 1 week before the final photography session.

2.4 Photogrammetry

2.4.1 Acquiring photos

Each coral polyp was photographed individually using a digital camera (Olympus tough TG-6 12 Megapixel) with the settings: Underwater Microscope, autofocus, and no flash. Additional lighting was produced using a Suptig 72 LED waterproof colour light shone through a silicon diffuser. The polyps were placed in the middle of a 22 l aquarium filled with filtered seawater (5 µm) and around 20 photos were taken around the fragment from two angles for the whole polyps, and from one angle for the split polyps (Fig 3B). In total between 32 and 44 pictures were taken of each whole polyp, and between 20 and 25 for each split polyp, on three separate occasions (one day post-fragmenting, four weeks post-fragmenting, and at the end of the experimental period). To ensure similar lighting on all photographs, tape was placed on the table to align both light and aquarium (Fig 3A) and corals were placed in an equivalent way at both measurements by aligning the yellow ID (Fig 1) in the same direction. For the final measurement, a Pasteur pipette was used to get rid of sediment on the substrate, remove bubbles from the coral, and to disturb the polyp in case its tentacles were still extended.

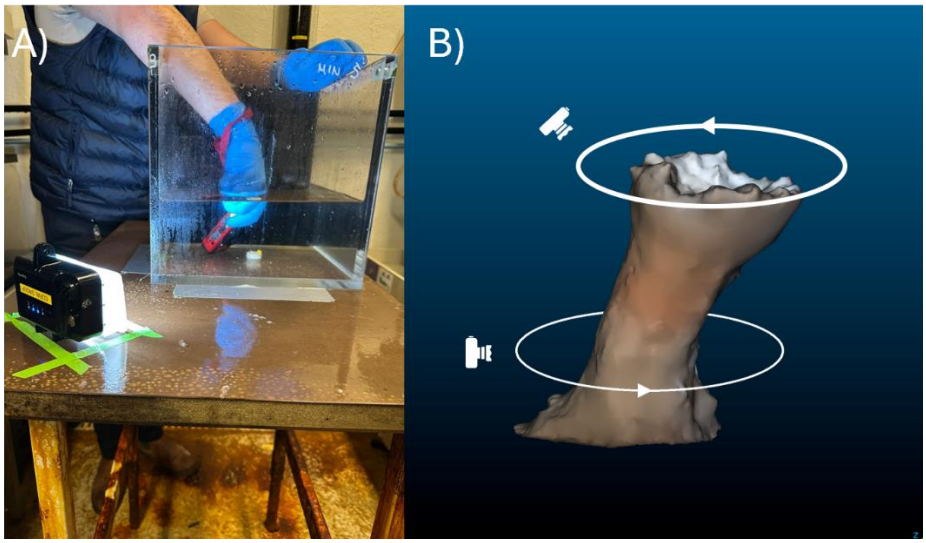

Figure 3 A) Coral and lighting placement inside the photography aquarium and B) average camera position during photography for the whole polyps. Photography angle of the split polyps was more in line with the lower circle.

2.4.2 Processing photos in Agisoft Metashape

To create three-dimensional (3D) models from two-dimensional (2D) imagery the photos were imported into Agisoft Metashape (standard edition 2.3.0) and aligned by matching features in the 2D images. The workflow in table 1 was adapted from Engström (2025), which in turn was adapted from Lange & Perry (2020) to function on the updated software and for usage of the batch process-function with all settings set to high. If any finished model were incorrectly created or excluded parts of the coral a new model was created according to the workflow in table 2. In summary, all images were sorted into individual folders based on which polyp the image was taken on and then each folder containing the pictures of one polyp were imported into Agisoft Metashape and sorted into individual chunks per polyp. The images were then aligned using high quality settings to generate tie points from which a point cloud was created, after which the depth maps contained in the point cloud were used to build the 3D model. All steps from alignment forward utilized the "batch process" function in the program to automatically process each polyp without further human interaction. Final polyp model was exported in PLY format (.ply) After batch processing the models were quality controlled and if the model contained unnecessary environment the model was removed and region resized to cut out excess parts of the point cloud. When the model was quality controlled the final model were exported in PLY format (table 2).

Table 1. Workflow used in Agisoft Metashape Standard edition v2.3.0 for constructing 3D models. The steps adapted from Engström (2025). Program was run on a 2019 MacBook Pro with 16 GB of memory and time is based on user experience.

Steps	Menu	Function	Action	Time (min)
Photo and chunk setup				
1	Workspace – left click Add Chunk	Add chunk	Add individual chunks for each replicate	5
2	Workflow – left click Add Photos	Add photos	Navigate to folder with photos, select and open	20

3	Workflow – left click Batch Process	Batch process	Add, apply to all chunks	1
				Total time: 26
Batch process		**Job type**		
4	Batch process – left click Add…	Align photos	Accuracy: High, Generic preselection, Key point limit: 120.000, Tie point limit: 12.000, Apply masks to: none, ok	1
5	Batch process – left click Add…	Optimize alignment	All "yes", except fit b1, fit b2, fit k4, ok	1
6	Batch process – left click Add…	Build point cloud	Source data: Depth maps, Quality: High, ok	1
7	Batch process – left click Add…	Build model	Source data: Depth maps, depth maps quality: High, Face count: high, Calculate vertex colors: yes, Reuse depth maps: yes	1
8	Batch process	ok	Run batch process	2248
9	Workspace – Right click chunk	Export	Export models in .ply format	10
				Total time: >2253

Table 2. Workflow in Agisoft Metashape v2.3.0 if 3D-models were not including the entire polyp or if the model was rotated incorrectly.

Steps	Menu	Function	Action	Time (min)
Removing and readjusting models				
1	Workspace – right click chunk	Remove models	Remove models	1
2	Model	Transform region	Resize region, change region to appropriate size, ok	3
3	Workflow – left click Build Model	Build model	Source data: Depth maps, Quality: High, Face count: High, ok	1
4	Workspace – right click chunk	Export	Export model as .ply	1
				Total time: 6

2.5 3D-model comparisons

CloudCompare v2.13.1 was used to calculate relative coral growth following the workflow in table 3, adapted from (Engström, 2025) which in turn was adapted from (Lange & Perry, 2020). The workflow and method were adjusted to the latest version of the program and by how I found the

same functions. MeshLab v2025.07 was then used to fill in holes in the meshes and to calculate the approximate volume of the whole polyp as shown in table 4. Both workflows and methods for CloudCompare and MeshLab, apart from some differences are almost identical to (Engström, 2025) and will be summarised below.

2.5.1 Processing 3D-models in CloudCompare

The 3D-models from model A (beginning) and B (end of test) were imported into CloudCompare where they were aligned based on four points occurring on both models. In my case the points were always the ends of the red measuring plastic and two points on the yellow ID number. After the models had been aligned with each other, the correct scale was obtained using the red plastic strip (5 mm) and dividing by the reference units from the measurement of the plastic strip:

$$Correct\ scale = \frac{5\ mm}{Reference\ units}$$

After calculating the correct scale, the new scale was inputted for both meshes to get their real-world sizes in mm. The red strip, ID number, and substrate were then segmented out leaving only the polyps for analysis. Utilizing the "compute cloud/mesh distance" function in CloudCompare, radial extension was calculated with model A as the reference and a gaussian statistical model (mean ± SD) was generated to estimate average growth in all directions. A scalar field was added to model B to visualize the growth patterns in assorted colours, showing where minimal to maximal growth occurred.

The relative volume growth was calculated using the surface area of model A from the 3D-model in CloudCompare. The surface area was then halved to compensate for measuring both the inner and outer surface area. The calculated volume was produced using this surface area and the mean growth:

$$Calculated\ volume\ (mm^3) = mean\ growth \cdot \left(\frac{Surface\ (model\ A)}{2}\right)$$

Table 3. Workflow in CloudCompare v2.13.1 comparing 3D-meshes, calculating radial growth, and visualizing where growth occurred. Steps based on (Engström, 2025), adapted from (Lange & Perry, 2020). Program was run on a 2019 MacBook Pro with 16 GB of memory and time per step is based on user experience.

Steps	Menu	Function	Action	Time (min)
Align 3D-meshes				
1	Main panel	Open files	Open directory with 3D-meshes (.ply) of same polyp from different times (A start, B finish).	1
2	DB Tree panel Main panel	Translate/rotate	Highlight 1 mesh, Translate/rotate mesh to align close to and in similar orientation as the second mesh.	3
3	DB Tree panel Main panel	Align meshes to scale	Align two clouds by picking (at least 4) equivalent point parts, choose points, adjust scale, align, ok	2

4	DB Tree panel Main panel	Get reference size (red plastic)	Highlight 1 mesh, point picking, select two points by reference and display, save	1
5	DB Tree panel Main panel	Adjust scale to mm	Highlight all meshes, edit, multiply/scale, change scale (x) to reference scale	3
				Total time: 10
Measure growth rate				
6	DB Tree panel Main panel	Segment only corals	Highlight both meshes, segment around outline of the models by polygonal selection, segment in/out, ok	4
7	DB Tree panel Main panel	Compute cloud/mesh distance	Highlight both meshes.segmented, compute cloud/mesh distance, choose model 1 as reference, uncheck signed distance, compute, ok	2
8	DB Tree panel Main panel	Scroll down	Highlight model B, display scalar field, check color scale visible	1
9	DB Tree panel Main panel	Measure surface	Highlight model A, edit, mesh, measure surface	1
10	DB Tree panel Main panel	Get radial extension and avg ±SD	Highlight model B, fit a statistical model on the active scalar field, distribution: Gauss	1
11	DB Tree panel Main panel	Save as .ply	Highlight segmented polyp, save current entity, save as PLY mesh (.ply), repeat for both segmented polyps	1
				Total time: 10

2.5.2 Processing 3D-models in MeshLab

Model A was then imported from CloudCompare into MeshLab to repair holes and calculate the approximate volume of the whole polyp. This was later used to calculate the percentual volume growth between model A and B:

$$Volume\ (\%) = \left(\frac{Calculated\ volume \cdot 100}{Volume\ (model\ A)}\right)$$

Table 4. Workflow used in MeshLab v2025.07 for closing holes and computing 3D-model volume. Based on the workflow in (Engström, 2025) with approximate time per step on the same hardware as in table 3.

Steps	Menu	Function	Action	Time (min)
Close holes and compute 3D model volume				
1	File	Import mesh	Navigate directory and select mesh B for the polyp, open	1
2	Main panel	Cleaning and repairing	Filters, repair non manifold edges, both methods, apply	2

3	Main panel	Close holes	Filters, remeshing, simplification and reconstruction, close holes, max size to be closed: 10 000, check: prevent creation of selfinteresting faces	1
4	Main panel	Compute volume	Filters, quality measures and computations, compute geometric measures	1
5	Main panel	Save	Export mesh	1
				Total time: 6

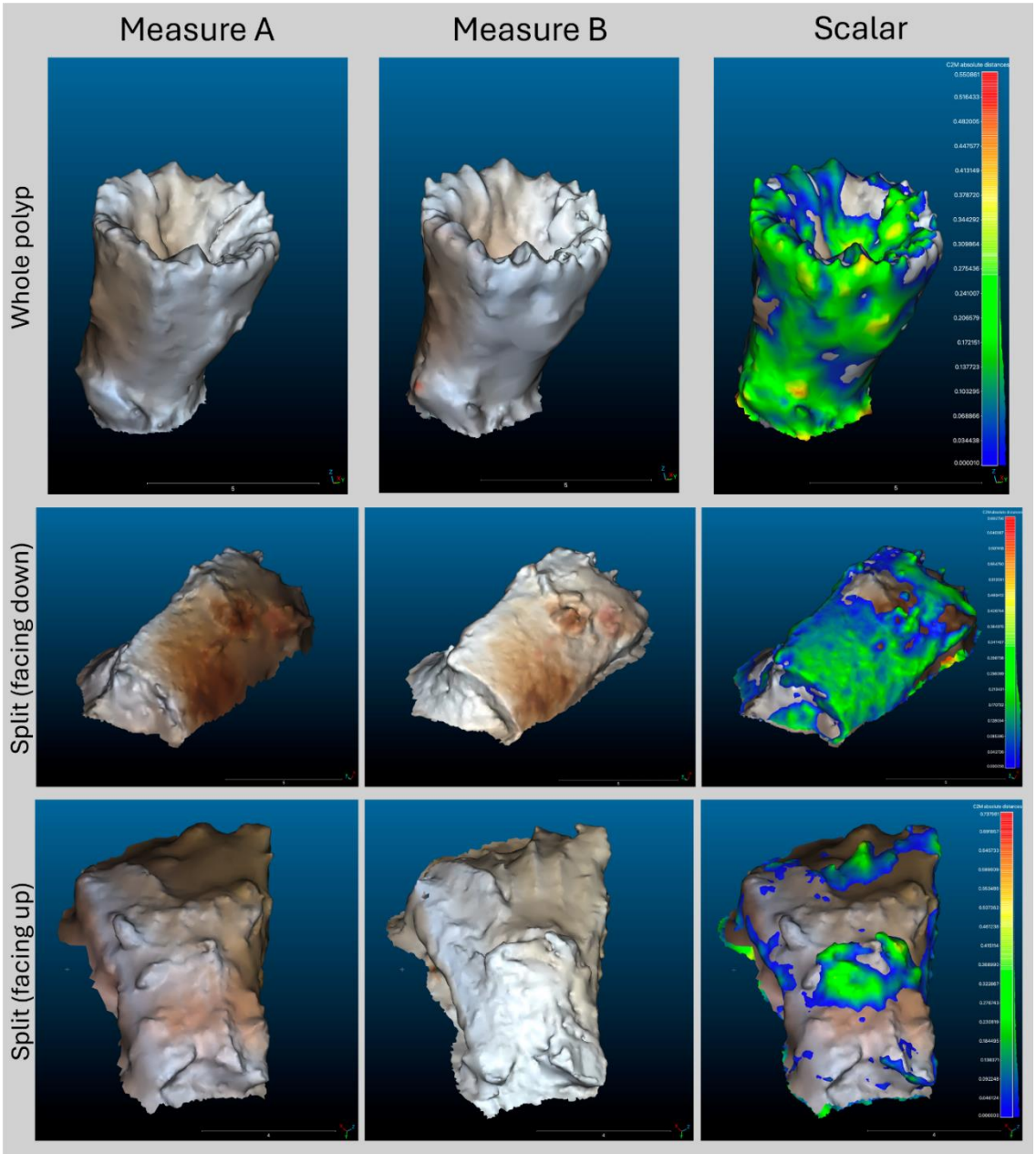

Figure 4: Workflow result in CloudCompare for one whole polyp and two split polyps with different rotations. Measure A and measure B were compared to each other with A as the reference. The scalar field shows where the polyp has grown and to what extent between the measurements.

2.6 Data analysis

The data was then loaded into RStudio (v. 2026.01.1+403; Posit team,2026) utilizing R (v. 4.5.0; R Core Team, 2025) for data analysis and to assess mean growth (mm), minimum growth (nm), maximum (mm), and volume growth (%). The data was first prepared by multiplying the minimum growth data by 1e^6 to get the growth in nanometres. After this the minimum growth data was log transformed and the percentual volume data was square root transformed to meet the assumptions of normality. Normality was then tested using Q-Q plots of a histogram and Shapiro-Wilk tests. Several one-way ANOVAs ($p<0.05$) were then conducted to evaluate how mean growth, percentual volume growth, maximum (MAX) growth, and minimum (MIN) growth was affected by substrate. If any significant effects were detected, Tukey HSD post-hoc tests were performed to evaluate the differences between the groups and interaction. For the split polyps, a nested multi-factor ANOVA was used to determine if feeding affected them in a significant way. Aquariums were nested into treatment since two aquariums were used for each treatment. Additionally, a linear mixed model was created to test if the split *D. pertusum* polyps were affected by aquarium as a random factor and another linear mixed effects model was created to test if colony origin had any effect on growth for the whole *D. pertusum* polyps.

3 Results

3.1 Substrate preference

In total 19 of the 20 polyps were turned successfully into 3D-models (except for polyp 14). This meant that all substrates had five replicates except for Portland cement, which had four replicates. The mean radial growth (Fig 5) and percentual volume growth (Fig 6). For the polyps across all substrates were extracted and quantified from CloudCompare v2.13.1.

3.1.1 Mean growth

Mean growth ranged between 0.100475-0.220573 mm for the ceramic plugs, 0.097449-0.317722 mm for the enriched ceramic plugs, 0.115776-0.249171 mm for the Portland cement, and between 0.142322-0.442658 mm for the metallurgic slag (Fig 5). The one-way ANOVA showed that mean growth was not significantly impacted by substrates (Table 5) and the linear mixed effect model with colony origin as a random effect showed that colony origin had no significant impact either (variance = 0) (Table 6).

Table 5: One-way ANOVA analysing the statistical significance of whole *D. pertusum* polyp mean growth on different substrates.

Mean growth	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**pr(>F)**
Substrate	3	0.01355	0.004516	0.55	0.656
Residuals	15	0.12319	0.008213		

Table 6: Linear mixed effects model analysing if colony origin could explain the statistical insignificance of mean growth in table 5. Note that the p-value for intercept (ceramic plugs) only indicate that the coral has grown significantly more than 0 mm.

Fixed effects	Estimate	Std. Error	Df	t value	pr(>|t|)

(Intercept)	0.14331	0.04053	15	3.536	0.00299
Substrateenriched_ceramic_plugs	0.02897	0.05732	15	0.505	0.62056
Substratemetallurgic_slag	0.07158	0.05732	15	1.249	0.23088
Substrateportland_cement	0.01991	0.06079	15	0.328	0.74779
Random effects:					
Groups	Name	Variance	Std.Dev.		
Colony	(Intercept)	0	0		
Residual		0.008213	0.09062		

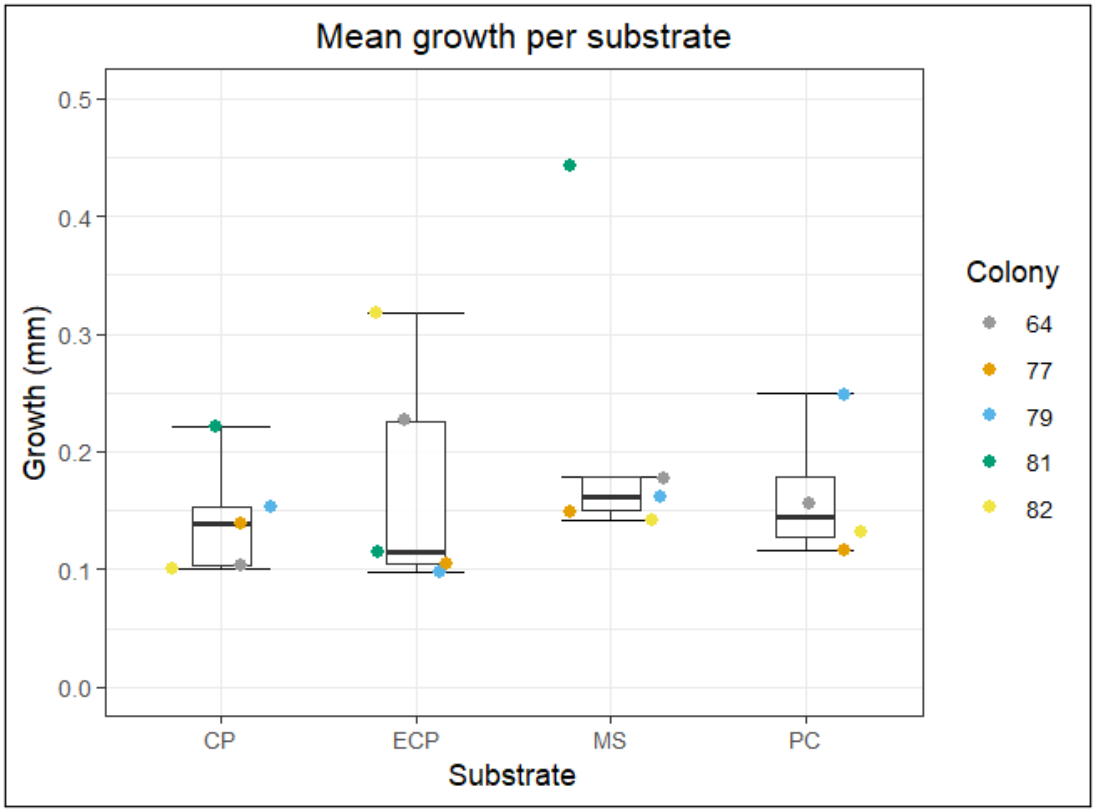

Figure 5: Mean growth in mm between the substrates ceramic plugs (CP), enriched ceramic plugs (ECP), metallurgic slag (MS), and Portland cement (PC). The boxplot shows the median (horizontal bar), interquartile range (box), and minimum/maximum values excluding outliers (whiskers). Sample size was n = 5 except for PC where n = 4. Each dot represents a polyp with the colour indicating the origin colony from which it was taken.

3.1.2 Relative volume growth

Relative volume growth was between 9.63-14.22 % for CP, 7.51-24.94 % for ECP, 8.88-18.25 % for PC, and between 9.8-30.6 % for MS (Fig 6). The one-way ANOVA showed that relative volume growth was not significantly impacted by substrates (Table 7) and the linear mixed effect model with colony origin as a random effect showed that colony origin had no significant impact either (variance = 0.03425) (Table 8).

Table 7: One-way ANOVA analysing the statistical significance of whole *D. pertusum* polyp percentual volume growth on different substrates.

Volume growth	Df	Sum Sq	Mean Sq	F value	pr(>F)
Substrate	3	66.1	22.05	0.486	0.697
Residuals	15	680.6	45.37		

Table 8: Linear mixed effects model analysing if colony origin could explain the statistical insignificance of percentual volume growth in table 7. Note that the p-value for ceramic plugs (intercept) only indicate that the coral has grown significantly more than 0 mm.

Fixed effects	Estimate	Std. Error	Df	t value	pr(>|t|)
Substrateceramic_plugs	3.3455	0.3751	14.9024	8.92	2.32E-07
Substrateenriched_ceramic_plugs	0.2927	0.5174	11.0782	0.566	0.583
Substratemetallurgic_slag	0.5834	0.5174	11.0782	1.128	0.283
Substrateportland_cement	0.2591	0.5501	11.6528	0.471	0.646
Random effects:					
Groups	Name	Variance	Std.Dev.		
Colony	(Intercept)	0.03425	0.1851		
Residual		0.66913	0.818		

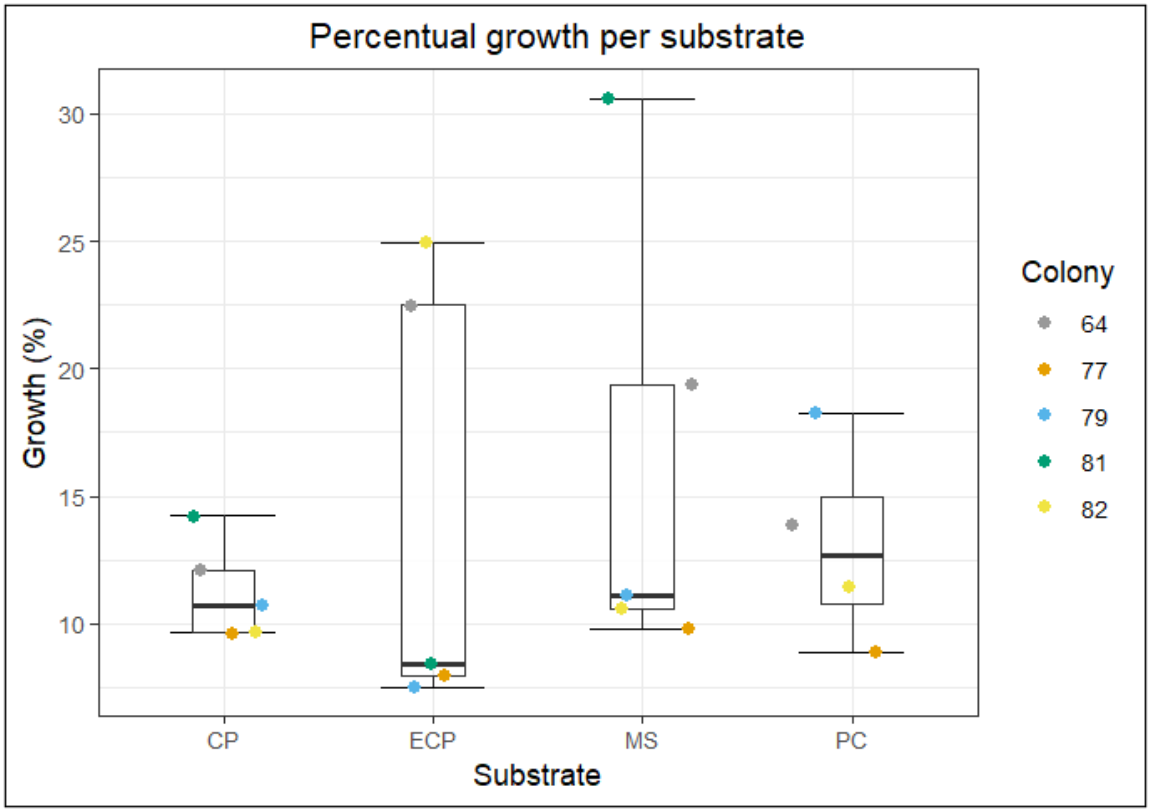

Figure 6: Relative volume growth in % between the substrates ceramic plugs (CP), enriched ceramic plugs (ECP), metallurgic slag (MS), and Portland cement (PC). The boxplot shows the median (horizontal bar), interquartile range (box), and minimum/maximum values excluding outliers (whiskers). Sample size was n = 5 except for PC where n = 4. Each dot represents a polyp with the colour indicating the origin colony from which it was taken.

3.2 Feeding effect in split polyps

Of the 24 polyps split and placed, a total of 20 were successfully turned into 3D-models (split polyps 7, 8, 12, and 23 were unsuccessful). Additionally, split polyp 24 died sometime during the experiment period but was left in since it showed some growth in the final comparison. This meant that a total of 20 split polyps were included in the final measurement with nine tissues face down and eleven tissues face up. Four of the tissue face down polyps were placed in aquariums with nutrients while the other five were starved. Of the tissue face up polyps a total of five were placed in the nutrient aquariums while the other six were starved. Mean growth for all split polyps was quantified and extracted from CloudCompare v2.13.1.

3.2.1 Mean growth

Mean growth in the different treatments varied between 0.148297-0.558317 mm for the nutrition-receiving polyps and between 0.122591-0.462066 for the starved polyps. The type 3 ANOVA of the linear mixed effect model (Satterwaite's method) shows growth was not significantly impacted by either food, rotation, or the interaction between them (Table 9), regardless of which aquarium the split polyp was in (Table 10).

Table 9: Linear mixed effects model analysing if treatment, rotation, or an interaction between treatment and rotation affected *D. pertusum* split polyp mean growth.

Mean growth	Sum Sq	Mean Sq	NumDF	DenDF	F value	pr(>F)
Treatment	2.9E-05	2.9E-05	1	2.2214	0.0015	0.9725
Rotation	0.00721	0.00721	1	15.5802	0.3722	0.5506
Treatment:Rotation	0.03924	0.03924	1	15.5802	2.0249	0.1745

Table 10: Linear effects model analysing if the random effect of different aquariums could have any effect on the *D. pertusum* split polyp mean growth. Note that the p-value for treatment food (intercept) only indicates that growth has occurred, not that it's significant.

Fixed effects	Estimate	Std. Error	Df	t value	pr(>|t|)
TreatmentFood	0.20179	0.072	7.04152	2.803	0.0263
TreatmentStarve	0.09247	0.09668	6.7445	0.956	0.3718
RotationUp	0.12841	0.09404	15.96	1.366	0.191
TreatmentStarve:RotationUp	-0.17976	0.12633	15.5802	-1.423	0.1745
Random effects:					
Groups	Name	Variance	Std.Dev.		
Aquarium	(Intercept)	0.000554	0.02353		
residual		0.01938	0.13921		

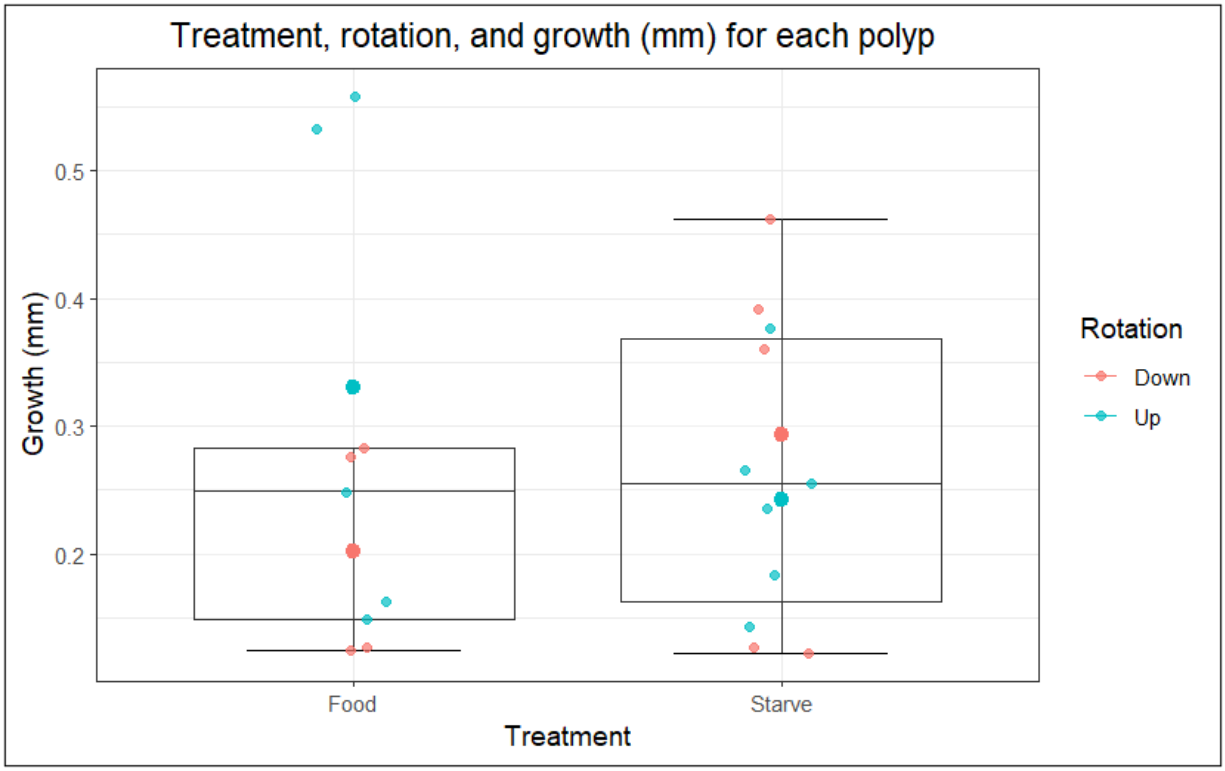

Figure 7: Mean growth of split polyps (mm) depending on which treatment they got and which way their tissue was facing. The boxplot shows the median (horizontal bar), interquartile range (box), and minimum/maximum values excluding outliers (whiskers). The larger dots represent the mean for the different rotations. Original sample size was n = 6, but after the experiment aquariums 1, 3, and 4 had n= 4, n = 5, and n = 5 respectively. Note that graph does not reflect the model exactly and is more to visualize the experimental structure and the raw data since treatment was confounded with the aquariums. Since there was no evidence of variance between the aquariums only treatment and rotation are visualized

3.2.2 Signs of survival and growth

After the final photogrammetry pictures were taken additional top-view photos were taken using an DSLR camera (Canon EOS 5D mark III 22.3 megapixel) of all split polyps. By eye you could see signs of survival on all *D. pertusum* split polyps through a stereoscope, indicated by live tentacles and that all polyps with tissue facing up had formed a new polyp head, except for split polyp 24 which did not have any tentacles or a new head forming (Fig 8).

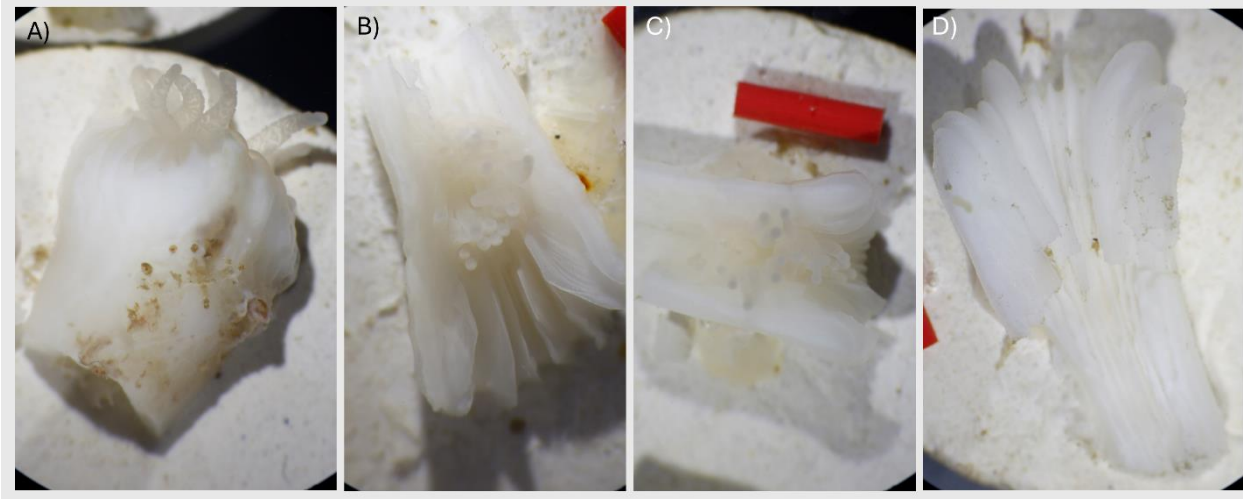

Figure 8: Photos of split polyps 3 (A), 14 (B), 15 (C), and 24 (D) all showing signs of survival with live tentacles except for polyp 24. Polyps 14 and 15 also have a new polyp head forming in the middle and polyp 3's head has started to turn upwards.

4 Discussion

4.1 3D-model and observational analysis

According to the photogrammetry of this experiment, the whole polyps mean growth ranged between ~0.09-0.32 mm and a percentual volumetric growth between ~7.5-30.6% % during the 10.5-week experimental period (~0.45-1.6 mm and ~37.5-153 % growth for 52 weeks respectively). The split *D.* pertusum polyps had a mean growth between ~0.12-0.53 mm and a percentual growth between ~7.2-24.4% (~0.6-2.65 mm and ~36-122 % growth for 52 weeks respectively) during the same time. While the average mean radial growth values are similar, albeit lower than the mean growth from Evelinas (2025) thesis, they do not align with other studies of *D. pertusum* growth that indicate a yearly radial mean growth rate of between 2.77-4.27 mm *in situ* and 25 mm *ex situ* (Brooke & Young, 2009; Rogers, 1999), which is several times higher. At the same time, the percentual volume growth from this experiment is too high to be realistic, since it should only be a growth increase of a couple of percent for the volume (A. Larsson, personal communication, April 29, 2026). When these results were obtained my supervisor and I discussed what could have made the results go awry. We assumed that it may have been a combination of bad models and that the data used for calculating the percentual volume growth using the surface area of model A for some reason was incorrect. Therefore, an attempt to calculate the volumetric growth of the models without the equations in 2.5.1 and 2.5.2 and instead solely comparing the volumes for both model A and B taken from MeshLab using the formula below was made, after closing the holes of model B according to Table 4.

$$Volume\ (\%) = \left(\frac{Volume\ (model\ B) - volume\ (model\ A)}{Volume\ (model\ A)}\right) \cdot 100$$

I did this for all polyps on the different substrates as a test and the result from these recalculations still seemed unrealistic. Therefore, it was agreed to disregard the new calculations without doing a full analysis and continue with the first calculations.

Even though the data from the 3D-models showed an unsignificant growth regardless of amount of nutrients or tissue rotation it was clear that all but one of the split polyps (polyp 24) had survived the experimental period, and since all fragments with their tissue facing up had formed new heads and tentacles (Fig 8) it was also clear that they had grown, some more than their models showed due to the above mentioned quality of the models. While it was difficult to observe any growth by eye on the fragments with their tissue facing down, you could see that the newly formed heads of the tissue-up fragments were mostly coming out of the centre of each fragment. This makes sense when this was often the part largest part with the most tissue still in place, giving the polyp access to left over nutrition pre-split to construct the new anatomy. When it came to the split fragments facing down, all were still alive, but none had produced a new head. Which indicates that it is harder for *Desmophyllum pertusum* to grow out of intact skeleton compared to growing out of the softer innards. These observations show that split fragments should be placed with their tissue facing upwards since it seems to be easier for the fragments to regenerate new heads and tentacles from this area. It also shows us that regeneration seems to begin in the centre of each fragment and on the damaged side of the coral which is consistent with previous studies of other slow growing

species, showing that smaller microfragmented corals grow quicker in the damaged parts of the tissue (Page et al., 2018). Still, something surprising was that fragment number 3's original head seemed to have started to migrate upwards (Fig 9). To my knowledge this is unusual behaviour for the species, and more research is required on how this is possible.

Figure 9: A close-up of split polyp 3, where the head of the polyp seems to have migrated a distance from the original position facing vertically to a position facing more upwards.

Based on the findings from the 3D-models, the data suggest that neither substrate choice or colony origin has a significant impact on the growth of microfragmented *D. pertusum* polyps and that nutrition availability has no significant effect either on the growth or survival of split *D. pertusum* polyps. These findings do not align with previous research on how growth speed is affected by different substrates or colony origin in tropical corals. These findings instead show that both colony origin and substrate placement has a significant effect on coral growth, with genet effect dwarfing the substrate effect. (Papke et al., 2021). However, the same paper also showed that the interaction between the genet and substrate did not have any significant effect on the growth, which strengthens the same findings in this study. It was also expected that coral polyps would grow faster on ceramic enriched with strontium and magnesium (Yus et al., 2024), or metallurgic slag since these materials have in the past proven to be good substrates for coral growth and larval settling (A. Larsson, personal communication, April 21, 2026). Even with my findings showing

otherwise compared to the previous studies those results seem to make the most sense. In theory the polyps should be able to absorb the minerals in the substrates in addition to their food to increase the coral skeletons growth, given that the glue attaching the polyp to the substrate is not too thickly applied and hindering the absorption. While glue could have been a factor for the differing results it seems unlikely since the same method of attachment has been successfully used in previous studies (Papke et al., 2021; Engström, 2025), and that only a conservative amount of glue was used for each fragment.

The boxplots in figure 5 show a generally even mean growth between the substrates with the median for MS being slightly higher than the median for both PC and CP, with ECP having the lowest median of the mean growth measurements. One could argue from this that there is no real advantage to any of the substrates and that you should pick whichever that is most available. However, the mean growth also considers both the largest and smallest growth for each model which may not represent the general growth of the polyp. For example: polyp 7 and 13 showed a maximum growth on a specific point to be 2.02 and ~2.45 mm and polyp 6 had a minimum growth of 0 mm. Both maximum and minimum values do not represent the entire polyp, but they may have skewed the results to be inaccurate depending on how large the area of maximums may have been. By looking at the boxplots in figure 6 give visual hints that both ECP and MS can potentially have a larger impact on percentual growth compared to CP and PC, with some polyps having grown much more compared to the rest. But this can also be interpreted as being errors in model measurement. When it came to the the median of percentual growth it is slightly higher for the PC compared to the rest which is similar to the study made by Papke et al., (2021) who found that growth speed in cement is slightly better than standard ceramic plugs. Both the mean and volumetric growth measurements could also have been influenced by a polyp's tentacles if they did not retract into the polyp when disturbed., thus confusing Metashape and CloudCompare to interpret soft tissue as the coral skeleton. When examining live corals this is always a possibility and the only way to completely eliminate this factor would have been to kill it and remove the soft tissue which also would have meant that no future growth measurements could be taken.

4.2 Method errors and limitations

Several of the errors and limitations have already been touched upon but the biggest limitations of this project in no particular order have been hardware, methods for measurement and feeding, and time.

4.2.1 Calcein staining

Calcein staining and staining in general is a method where the corals are put in a solution of either calcein ($C_{30}H_{26}N_2O_{13}$) and bicarbonate ($NaCHO_3$), or other staining agents for several hours before being placed in their long-term aquarium. At the end of the experimental period, you take the coral fragments out of their aquariums and transfer them into freshwater to kill them. When the fragments are dead and only the skeletal structure remains, the newly formed skeletal structures will appear in the natural off-white colour common to the species, while the stained parts will be fluorescent/coloured depending on staining agent, which enables researchers to make qualitative observations of exactly where and how the coral has grown (Engström, 2025).

In the original project plan the intention was to include fluorescent calcein staining at the end for analysis not just for the split polyp fragments, but for all polyps in the experiment. When the

time came to create the calcein mixture it was discovered after adding half of the calcein required for the solution that there was no more of the substance at TML. By this time the solution was unrecoverably diluted and unusable and delivery times for new calcein were too long. Due to this, I had to abandon the calcein staining after waiting to start the experiment for a week and rely solely on the 3D-model measurements, for fear of the corals not growing enough to be able to measure a difference. For any future studies it would be beneficial to use some form of staining for qualitative analysis since it could compliment the quantitative results of the photogrammetry.

4.2.2 Photogrammetry

When I began to form the experimental design for this project, much of the methodology was based on what Engström (2025) had done previously and what worked for her. After discussing the methods of photogrammetry with my supervisor, we agreed that a total of 40 images of each whole coral polyp and 20 images of the split coral fragments should be enough for the program. This differed from Engström's methods, where the polyps received 40 images from each angle for a total of 80 images. After building the models it looked like it would have been beneficial to take more photos of each coral, especially for the split polyps. While the 3D-models for the whole polyps in general were adequate, some lacked detail or even parts that you could see with the naked eye. There was a stark difference in quality of the first 3D-models for the split polyps was clearly worse compared to the final measurement, often being darker, less detailed, merging with the ID-tag, or showing a figure not accurate to the real world, sometimes showing inward growth when it was model A's lack of detail that extended outward where no coral was (Fig 10). Due to my inexperience with the programs used for the project there was nothing I could compare the first measurement with and took for granted that all models were going to be similar. In the end I had to use the models that were rendered and did to the best of my ability try and segment out any unnecessary mesh from the models to get an as accurate comparison as possible.

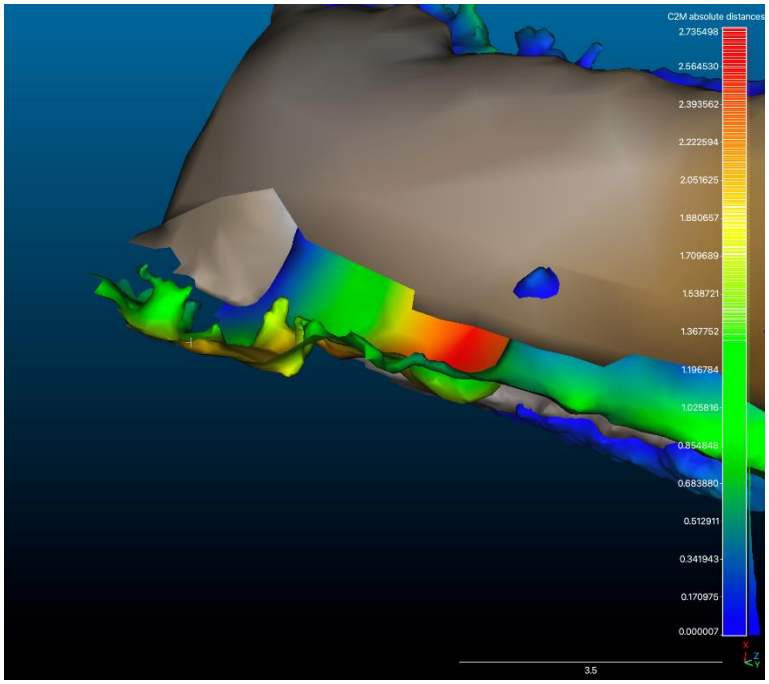

Figure 10: An example of a bad model from the split fragments. While the final measurement (scalar) was sufficiently detailed, the first model (grey) was not and lacked both surface detail and created an illusion that the final measurement had grown inwards compared to the first model.

Since Agisoft Metashape works by stitching together 2D-images to form a 3D-model, more images could have meant more material to stitch together while leaving room to exclude photos of worse quality, which could have led to better models. Additionally, images could have been improved with better photography techniques and positions. To start, the camera used for the pictures (Olympus tough TG-6 12 Megapixel) had its lens in the middle while the shots were taken vertically. This resulted in most of the lower-angle pictures being slightly higher than if the pictures had been taken horizontally. There were two reasons for not shooting the pictures in horizontal mode. The first reason being that it required two hands for me to keep the camera steady while shooting. This was difficult because the table used during the photography sessions was too small for me to comfortably keep hold of the camera. Had the table used been some decimetres lower it would have been easier to take the pictures comfortably. The second reason for not shooting horizontally was because I could not see what the camera was capturing with the screen level to the sides. By having the camera at an angle, it was possible to not only see what was captured but also to confirm that the camera had taken a picture, since there was no audial sign from the camera that a photo had been taken. In the future, a good practice would also be to put the substrates on a heightened elevation to be able to capture photos from underneath the split polyps, since it was noticed that much detail on the underside of the heads and sides were lost in the modelling. This would also be beneficial since horizontal photos could be possible with heightened substrates.k

4.2.3 Nutrition

While there was no indication of nutrients influencing polyp growth, it can also be explained by the amount of nutrients the corals received. The split polyps were either starved or fed with an amino acid solution (AMIN) as the only food source for the duration of the experimental period. The fragments that were fed got a dosage that was over twice the recommended amount of 0.5 ml/100 l (0.25 ml/12 l). However, since these nutrients were designed more for corals with zooxanthella that can produce their own food using photosynthesis AMIN is meant to be used as a compliment to tropical corals than as a primary food source. Because *D. pertusum* lack zooxanthella and are unable to produce their own energy, the initial experimental design planned to combine AMIN nutrients with MIN S, which consist of natural nutrients mostly from solved copepods. This alternative was forgone when I realized that the slurry was too thick to accurately measure for the tiny dosages needed in the small aquariums (recommended dose was 0.3 ml/250 l). Instead of upping the amount of AMIN used I decided to keep it at the same amount, but it may have been beneficial to feed them with a higher dosage of nutrients to counteract the missing nutrients.

4.2.4 Hardware and program requirements

The next improvements I would recommend for future studies is to use improved hardware capable of running Metashape to its largest extent. To render my models, I had access to my supervisors' laptop (macintosh) with 16 GB in RAM while the minimum requirements to run Agisoft Metashape (v.2.3.0) was 32 GB in RAM. This required the computer to run for longer, for lesser-quality models using the batch process function. In total, the batch-process rendering of the models took ~42 hours for the first measurement and ~62 hours for the second measurement. For comparison, an additional experimental batch process was made of the first measurement using the same workflow, but with all settings set to the highest possible quality. This rendering took ~137 hours (5.7 days) which simply wasn't a feasible time to wait for the final measurement. Batch processing,

while an especially useful function for this project also left out a couple quality assurance functions that could only be accessed by rendering each polyp individually. Unlike me, Engström (2025) had access to a more powerful computer, enabling her to render each model individually at the highest quality and in a timely manner. This in turn gave her better models with less errors and more realistic data to analyse. In the future it is highly recommended to use a computer with at least the minimum requirements for the programs and to individually model each polyp for quality assurance.

4.2.5 Time

In the end the largest limitation for this study was time. *D. pertusum* grows slowly which meant it was important to let the corals grow for as long as possible, while still leaving room to do the analysis. With the waiting time for the calcein delivery and the discovery of the extended time it would take to render the coral models, the timeline of the project had to be adjusted in line within the new limitations. The original plan was to let the corals grow for 12 weeks with a week of acclimatizing before measurement. This had to be shortened to 10.5 weeks with no time for acclimatizing to the new aquarium which could have impacted the results gained from both mean growth and percentual growth. Another time-sensitive limitation was found after the experimental period. A similar study made on tropical corals stated that Portland cement must be soaked for approximately six weeks before becoming chemically inert and ready to use (Papke et al., 2021), Pre-soaking the cement could also have given different results but the waiting time for this would also have been too long. Should another experiment of substrate effect take place, pre-soaking the substrates should be taken into consideration in case it might yield different results. When it came to the measurement of volumetric growth, another method of calculation was explored after the results from the first method had been analysed in RStudio using only comparing the models in MeshLab. While these results were still unrealistic, they may have yielded other results compared to the results from the first method of calculation volumetric growth. Lastly, the models of the split polyps from measurement two and three could have been compared to each other. Unlike the models from the first measurement, the models from the second measurement were more detailed overall, including underneath. However, the process in CloudCompare, MeshLab and result analysis from RStudio would have taken around two more days to complete. This could potentially have benefitted the results when a better alignment and segmentation of the models would have resulted in more accurate estimations of mean growth and volumetric growth. The drawback to this was that the shorter time between the comparisons also could have had an effect on the results. If a continuation study were to be made of the same corals, it would be preferable to use the second measurement of the split fragments as the reference models for any future comparisons. Other than more detailed models, this would also be beneficial since the four weeks lost could be seen as an acclimatization period.

4.2.6 Conclusion

In summary, this study's primary aim was to investigate if the mean and volumetric growth of *Desmophyllum pertusum* polyps is affected by either the substrate it is placed upon or the colony origin of the polyp. The secondary aim was to investigate if split fragments regeneration and survival was affected by nutrition amounts or tissue rotation. In the end no statistical significance was observed in either aim but it was observed that all but one split fragment had survived and that all split fragments with their tissue facing up had grown a new head and tentacles in the middle of

the fragment, except for the one dead fragment. Despite the statistical results from this study, the methods used in this study have successfully been applied in previous studies to show both that photogrammetry is a valid and beneficial way to measure corals (Lange & Perry, 2020), and that both colony origin and choice of substrate can have significant effect on coral growth (Papke et al., 2021). There were several points in this study that could have been improved upon, from additional measurement tools like calcein staining and better hardware for model rendering, to simply giving the coral more time to acclimatize and grow. While none of the results in this study have proven to be significant the study could be used as a stepping stone for future research in the area, and if the recommendations for the better methodology are followed, more accurate and perhaps even significant results may be reached.

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