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Eco-physiological responses of cold-water corals to anthropogenic sedimentation and particle shape

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Natural Resources Management
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Acknowledgments

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Sincerely.

Stephanie Liefmann

Abstract

Cold-water coral habitats have been known to science for centuries, but only recently have scientists stated to study them. Among these habitats, also soft corals are found, and they have been even less investigated than the more well known stony corals. Some of the aspects that are known about these habitats is that they have very slow growth rates, they are also associated with high species-diversity and they are sensitive to anthropogenic disturbances. One anthropogenic disturbance that might occur is excessive sedimentation, from activities such as mine tailing disposal to the marine environment by the mining industry. Particles contained in the tailings are sharper than natural occurring sediments, thus the morphology of the particles could also affect soft corals. Two soft corals: *Duva florida* and *Primnoa resedaeformis* were chosen as model organisms to study the effects of excessive sedimentation and the corresponding effect of particle shape. Corals were exposed to a sediment concentration of 18 mg L^{-1} of two type of sediment.The first type was rough edged mine tailings (MT) and the second, smooth edged spherical glass beads (GB), both with particle size distribution was 0-63µm. The experiments lasted for 3 months. Sedimentation effects were investigated using $^{13}C/^{12}C$ isotope ratio to assess food intake, effects on the tissue and behavior were determined with time lapse pictures, and histological samples to enumerate and identify particles inside the polyps. Food intake decreased significantly in *D. florida* and increased significantly in *P. resedaeformis*, exposed to mine tailings. *Duva. florida* exhibited a behavioral response with the whole specimen being contracted for longer periods of time under MT treatment. *Primnoa resedaeformis* lost significantly bigger proportion of polyps than control individuals under both mine tailing and glass bead treatments. Histological samples showed only mine tailing particles of sizes between 0-10µm embedded in the tissue of both species. The results suggest that sharp small particles are more harmful than smooth edged particles. The results of this study could aid to create guidelines for management of cold-water soft coral habitats in regard to anthropogenic sedimentation disturbances.

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2 Introduction

Cold-water coral reefs have been known for centuries (Rogers, 2004), but it is not until recent years that technology has allowed the study of these fascinating habitats. Thus no many studies have been done on their ecology and physiology, and most published paper have focused on one species, the stony coral *Lophelia pertusa*. Extensive studies have been done in the Norwegian reefs (Järnegren & Kutti, 2014; Mortensen, Hodnesal, & Thorsnes, 2015) and the USA has also undertake nextensive study and exploration of these habitats, (for further information see deepseacoraldata.noaa.gov (NOAA, 2016)) .Canadian cold-water corals habitats have also been monitored to some extent, (Gilkinson & Edinger, 2009). Soft Corals, (Alcyonacea), living in cold-water ecosystems have been given very little attention. The presence of soft coral habitats is important for the benthic community because they can act as ecosystem engineers (Scinto et al., 2009) and harbor a number of associated fauna (Krieger & Wing, 2002; Mortensen & Mortensen, 2005). It has been documented that these ecosystems are very vulnerable to human impact such as; trawling, hydrocarbon exploitation, mine tailing disposal, deep sea mining (Ramirez-Llodra et al., 2011) and references therein; and fast changing environmental conditions related to global warming (Fabricius & De'ath, 2001). Paradoxically, the eco-physiological responses of soft corals to environmental key variables (eg. light, temperature, salinity and food), and stressors produced by human activities such as increased sedimentation and water temperature have not been documented in detail.

The polyps of soft corals polyps have eight tentacles, explaining their name octocorals. Each tentacle is covered by pinnules, they do not rely on hard exoskeleton, but instead they have sclerites (calcium carbonate spicules) to support their tissue, and many taxa (gorgonians) secret a central axis made of a calcified material and gorgonin (Complex protein containing appreciable levels of Br, I, and tyrosine) (Fig. 1A). Each polyp lies in a gelatinous matrix called the coenchyme, which is penetrated by a tube system for inter-polyp communication (Fig. 1 A, B). The polys are retractable but part of the polyp is still exposed when compared with a retracted hard coral polyp inside the theca.

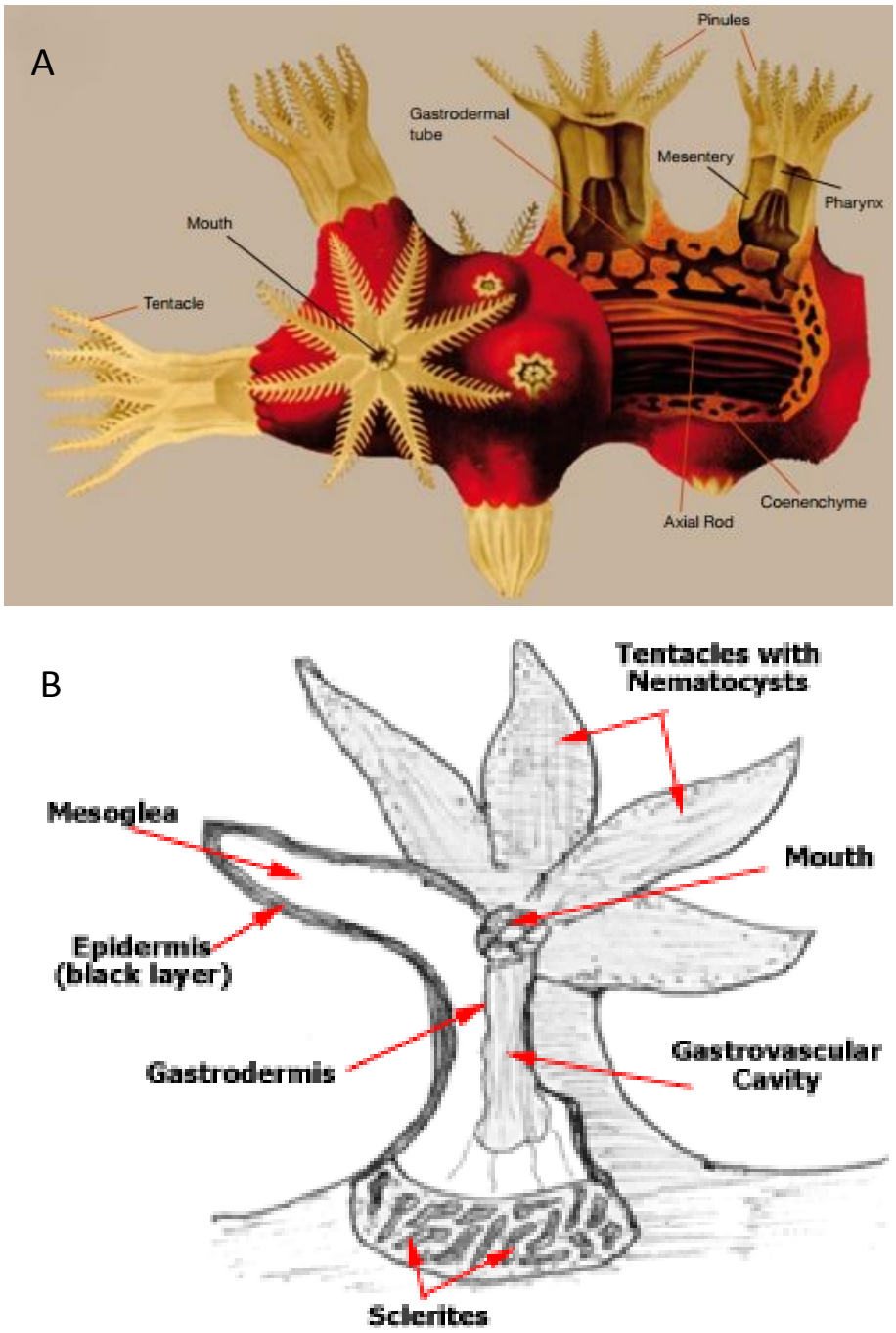

Figure 1. A: Structure of a gorgonian and gorgonin axis. Figure from plate collection Berlin university, modified by Ofwegen and Cairns (2010)

B: Anatomy of a soft coral polyp. The epidermis contains specialized cell such as. Nematocyst, epitheliomuscular cells, sensory and primary nerve cells, mucus producing cells, and interstitial cells. The gastrodermis has also a number of specialized cells and structures glandular cells, epitheliomuscular cells, mesenterial filaments.

Figure courtesy of http://www.peteducation.com/article.cfm?c=0&aid=2987

2.1 Studied anthropogenic disturbance

One anthropogenic disturbance is the deposition of mine tailings. It is defined as the waste remaining after the extraction of the mineral of interest from the ore by means of crushing, and milling. Waste specific contents depend on the extracted mineral (Ramirez-Llodra et al., 2015). Several tailing deposition methods exist : Riverine discharge, was widely used but now just four mines in the world use this method (Vogt, 2013).Mine backfilling, filling back the abandoned parts of the mine with the waste material. The great majority of industrial mines (99.4 %) deposit their tailings in artificial dams, impoundments or lakes (Vogt, 2013). The problem with this type of disposal is that they represent a health hazard for nearby organisms and people (Cornwall, 2013). Such disposal areas can be aesthetically unpleasant. Land disposal competes with other usages of the land such as agriculture and recreation.(Sierralta, 2014; Vogt, 2013)

Landfills and dams also represent a big management endeavor, and adequate geological conditions have to be met for them to be an option(Cornwall, 2013). Dam failures have long lasting environmental and societal consequences (Arnesen, Bjerkeng, & Iversen, 1997; Koski, 2012; Martin & Davies, 2000; Sammarco, 1999) A recent example the failure of the Samarco dam in Bento Rodrigues Brazil the 5th of November 2015.

To avoid the possible complications related with land tailing deposition, discharge at sea is also an option. Three forms of deposition normally occur at sea: Coastal/shallow water tailing disposal (CTD), deposition takes place in coastal areas, and often in the productive euphotic zone (Franks, Boger, Côte, & Mulligan, 2011). Submarine tailing disposal (STD): is deposited through a pipeline shallower than 100 m but under the euphotic zone. Deep submarine tailing disposal(DSTD), is deposited out deeper than 100 m depth with final deposition below 1000 m.(Ellis & Ellis, 1994; Skei, 2014; Vogt, 2013). The idea with DSTD and STD is that the slurry (tailings and water mix) does not mix with the surface water and to avoid plumes, hence coagulant and flocculent agents are used. When using these techniques, the accepted consequence is that all the biota under the dumping site will be lost (Vogt, 2013). The loss will have consequences for the adjacent environment, and those depending on finding food sources in this area (Vogt, 2013). Little information

is available on deep-sea ecosystems regarding the biology and dynamics(Armstrong, Foley, Tinch, & van den Hove, 2012) making it difficult to assess the exact impact of mine tailings. As pointed out by Vogt (2013) resuspension of particles by upwelling events and/or current systems can also spread the tailings to wider areas. Also particles smaller than 10µm remain in suspension for longer periods of time (Syvitski, Asprey, Clattenburg, & Hodge, 1985) and can disperse away from the designate dumping site, causing problems for nearby biodiversity because of excessive turbidity and sedimentation.

Norway is one of the few countries where STD are permitted, 7 mines use this practice, and two others are pending for permission (Kvassnes & Iversen, 2013). Active mines deposit between 30 000 to 3 million tonnes of tailings a year (In Norway). In 2013 none of the tailings contained any significant concentration of potentially toxic metals such as copper or lead (Kvassnes & Iversen, 2013).Thus only the physical consequences of the tailings on the nearby biota are expected such as smothering by burial, system clogging tissue abrasion. As stipulated by Kvassnes and Iversen (2013), the sharpness of the tailing particles can also have an effect on corals, considering that particles from mine tailings have rougher edges than natural occurring sediments (Gray, 1974). Soft corals exposed to plumes of tailings outside the deposition area are possibly vulnerable to harmful effects such as system clogging as documented for sea sponges (Tjensvoll, Kutti, Fosså, and Bannister 2013),tissue abrasion as shown by Riegl (1995) and higher metabolism due to stress and supplementary mucus production as demonstrated for scleractinian and alcyonacean tropical corals (Riegl & Branch, 1995)

This study aims to understand the effects of shape and concentration of mine tailing particles on cold-water soft corals. My main hypotheses are:

- Cold-water soft corals respond to realistic exposure of mine tailings with respect to tissue abrasion and metabolism.
- Different species of cold-water soft corals responds differently to realistic exposure of mine tailings.
- Cold-water soft corals respond differently to mine tailings and sediment with spherical shape with respect to tissue abrasion and metabolism.

To prove these hypotheses, two species present in Norwegian waters, the Cauliflower coral and Red tree coral were chosen as model organisms. The Cauliflower coral, *Duva florida* (Rathké, 1806) is found in the North Atlantic along the Norwegian coast, Hudson strait, Davis strait, from 40 to 1500 meters deep. It typically grows on rocky substrate, often attached to *Lophelia pertusa* gravel. The gorgonian the Red tree coral, *Primnoa resedaeformis* (Gunnerus, 1763), is typically found in the North Atlantic at depths between 40 to 1000 m. Colonies can grow more than 1 meter in height and are usually found close to *L. pertusa* aggregations. Soft corals were chosen as very little information exists about this group in general. *Duva florida* and *P. resedeaformis* where chosen as they represent two different structures of soft corals (one with internal axis and one without). This study also aims at elucidating the basic biology of these two species. The two coral species were aimed to be exposed to sedimentation rates of 50 mg $^{L-1}$ with non-reactive particles of different shape in size range from 0 to 63 µm. To obtain knowledge of mineral particles as a stress factor for metabolism, food intake was assessed by ^{13}C isotope enrichment of food. Tissue condition was monitored with pictures and histological samples. The information gathered in this thesis will hopefully be of importance for future environmental monitoring programs and for management decision making.

3 Materials and methods

3.1 Specimen collection.

The cauliflower coral *Duva florida* (Rathke 1806) collection took place the 21th of May 2015 at Nord-Leksa (DMS coordinates 63° 36' 402" N, 9 ° 23' 145" E) outside Trondheimsfjorden (Fig. 2). The sampling was done from the NTNU research vessel Gunnerus using a remotely operated vehicle (ROV) Sperre Sub-fighter K-30 equipped with a manipulator arm and sampling net (Fig. 3).The corals were scooped from the gravel using the sampling net. All individuals where brought from between 174 to 188 m depth. The ROV dived in total 6 times and after each dive the living corals where collected from the ROV-sampling net and carefully placed in temperature isolated containers filled with 10 ° C deep-water for transportation to the laboratory facility at Trondheim Biological Station (TBS). The corals remained in the containers for 8 hours. At TBS, the Cauliflower corals were transferred to three 108 L containers with deep-water (water from 100 m depth, filtered through sand-filters and at a temperature of 8 ° C) flow through system of 20 ml sec^{-1},with this set-up the water was exchanged in the tanks 10 times daily. The specimens were left to acclimate for two weeks.

Red tree coral *Primnoa resedaeformis* (Gunnerus ,1763) colonies were sampled in the same manner as the Cauliflower coral, but instead of being scooped they were snapped close to the base using ROV manipulator arm , and then placed in the net. Collection took place the 26th of June 2015 from Tautra (DMS coordinates 63° 34' 205" N, 10° 25' 512" E) (Fig. 2), from 94 to 113 m of depth. Three large colonies where collected, in order to fit them in the temperature isolated containers they were fragmented in half. Upon arrival to the lab the colonies where placed in a 1764 L tank hanging from the basal end to avoid tissue damage. The tank was equipped with deep-water flow through system of 100ml sec^{-1}, (water was exchange 4 times daily). Colonies were left to acclimate for 3 days.

Figure 1: Map showing the places in MID-Norway where the corals were collected, marked by a yellow pin. Nord-Leksa (63° 36' 402" N, 9 ° 23' 145" E) location for the Cauliflower corals, and Tautra (63° 34' 205" N, 10° 25' 512" E) for the Red tree corals.

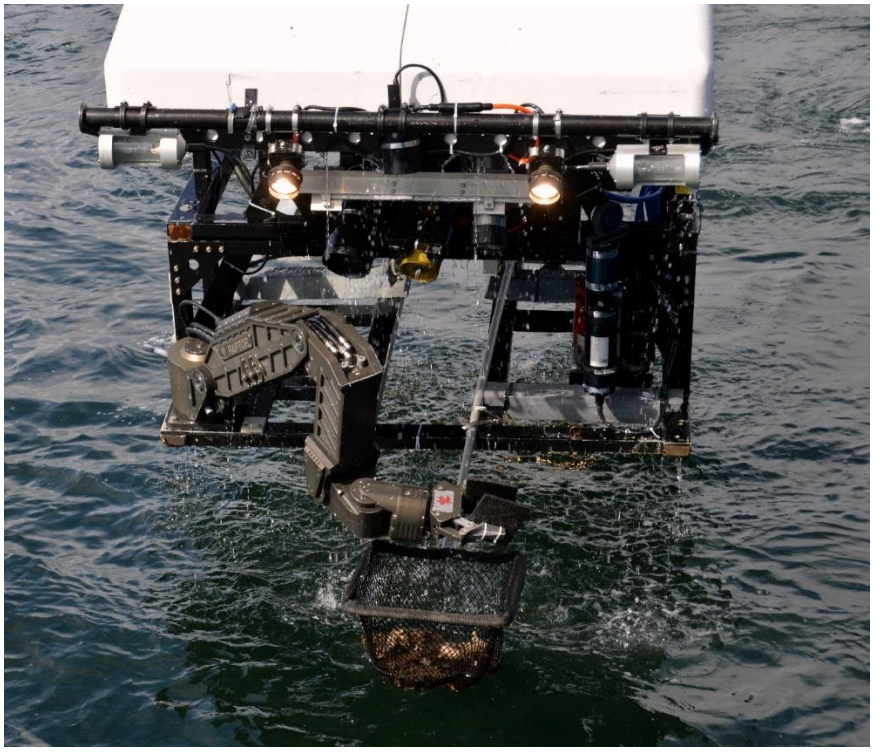

Figure 2: Remotely operated vehicle (ROV) with sampling net, and manipulator arm used for coral picking.

3.2 Maintenance of Corals in the lab

On the 2nd of June 2015, *D. florida* specimens were transferred to nine different 46L aquaria. Individuals were gently detached from the dead *L. pertusa* fragments and the epifauna on them. Each individual was placed in a glass plastic support (Fig. 4A) to mimic the support provided by the coral gravel. On the 29th of June 2015 *P. resedaeformis* specimens were fragmented from the colonies and put in supporting holdfast made of Reef construct cement, plastic and glass (Fig. 4B). It was ensured that the fragments in the same aquaria came from different colonies. The fragments were added to the aquaria the 29th of June 2015, three specimens in each aquarium. Flow through system provided deep-sea water from the Trondheimsfjorden from 100 m depth. Flow was maintained at 4ml sec^{-1}, meaning an exchange rate of 345.6L in a 24h period. Each tank was equipped with a MICRO-JET MC 450 pump to create water movements. The aquaria were maintained in a cold, dark temperature controlled room where temperature was kept between 6-4° C. Temperature was measured twice a week. Corals were fed twice a week with 6.7 ml of *Artemia salina* nauplii, final concentration per aquarium was 0.145ml nauplii L^{-1} .

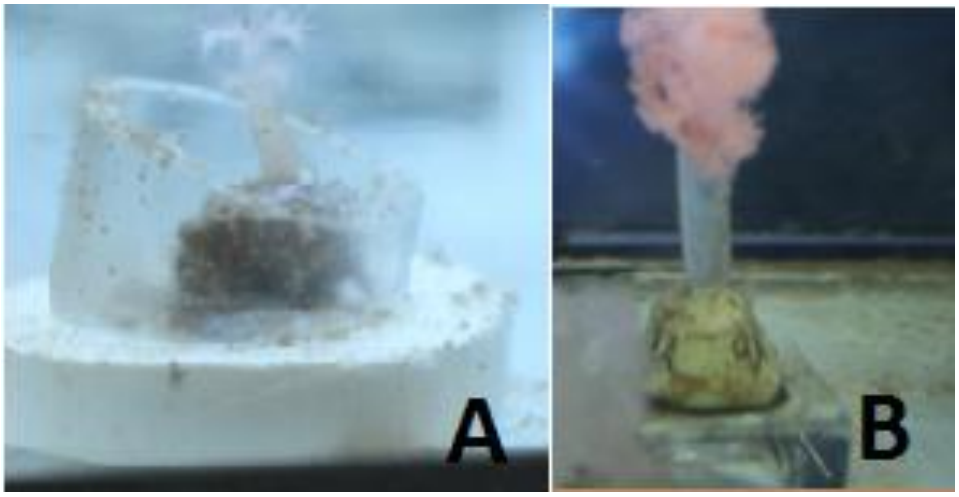

Figure 3A Plastic support for *D florida*. 4B: Reef construct support for *P.resedaeformis*

3.3 Experimental design

3.3.1 Time-lapse pictures.

In order to document polyp activity, feeding behavior, and learn more about the general biology of the two species, time-lapse pictures were taken. A Canon EOS D5 Mark II digital camera with a macro objective, white balance was set to artificial light in AV mode, and a Sigma Electronic Macro Flash EM-140 DG was used for time-series picture of corals in experimental aquaria.

The camera was mounted on a tripod, and placed in front of each aquarium for a period of 24 hours. The camera was controlled by an external timer to take a picture every hour.

Two time-lapse photo series were carried out. The first one from 18-26th of June 2015 (one day per aquarium), with the Cauliflower corals. The second time-lapse study was performed 16- 23th of July 2016 with both species.

During feeding, the corals were closely observed, and pictures taken when appropriate in order to document feeding ecology.

3.3.2 Sedimentation.

The corals where exposed to two treatments, mine tailings and glass beads, where the sediment concentration in the aquaria was aimed to be 50 mg L^{-1}. This concentration was chosen because it was the mean value of re-suspended particles after fish-trawling as calculated by de Madron et al. (2005). The "hazardous concentration for 5% of the population" (EC5) for barite drill cuttings was found to be 17,9 mg $^{L-1}$ calculated by Smit et al. (2008). Thus the chosen final concentration of particles in the aquaria should be ecologically relevant.

Mine tailings Treatment (MT): One of the two minerals used in the experiments was mine tailing sediments from Rana Gruber, an Iron ore mine situated in Mo i Rana Northern Norway. The mine tailings were of the same origin as used in (Haugland, 2014) comprising mainly quartz, muscovite, chlorite and magnetite. No evidence of

toxicity in the tailings was found, the particles had a density of 1.5 g cm^{-3} (Haugland, 2014). The tailings were dried by the geological department in NTNU and sieved through a 100µm mesh. Later it was re-sieved to achieve a particle distribution from 0 to 63µm. Sieving was done using a Endecotts test sieve shaker 2MK11 through a 63 µm Endecotts sieve. The second sediment treatment was Glass beads (GB) (purchased from Oberflächentechnik Seelmann trough eBay) ranging from 0 to 63µm. The glass beads were mostly composed of SiO_2 and NaO_2 and had a density of 1.6 g cm^{-3}. Each treatment had three aquaria (replicas), and three aquaria were not exposed to any treatment and used as control.

Both types of sediments were observed under a Leica stereo microscope using a M205C lens at magnification X10. Particle size distribution was measured using Zeiss Zen Widefield 2012 software. Five samples from each sediment type were mounted into a slide and the particles where circled on the computer screen as to assess their diameter with the help of the software.

Sedimentation treatment; a 40-liter conic tank contained a sediment solution of 200mg $L^{-1}$of ether the Mine tailing or Glass beads. Each system was equipped with a Oceanrunner 1200 (AB Aqua Medic GmbH, Germany) mixing pump to keep particles in suspension. An electromagnetic dosing pump (Iwaki EWN-B31VCR) distributed the sediment solution to each of the aquaria (Fig. 5). One pump distributed sediments to three different aquaria to keep a constant sediment concentration of 50mg L^{-1}. The delivery system of the pumps to achieve the desired concentration was calculated using a spreadsheet based on the model described by (Anthony, 1999). Each sediment dose pump was set to work in intervals of 1 minute on 5 minutes off, delivering 200 ml min^{-1} divided in 4 separate hoses (Fig.5). One of the hoses returned to the sediment bucket since each treatment had three replicates. The hoses were rotated among aquaria having the same treatment (once a week). The cycle ran for 6 hours, followed by a 6 hours pause in order to mimic semidiurnal tidal activity of the Trondheimsfjorden (Sakshaug & Sneli, 2000).

The sediment slurry lasted for 2 days, after which the tanks were rinsed rigorously during the 6 hour off cycle, to avoid bacterial growth, then refilled. Sedimentation experiments started the 12th of January 2016 and concluded the 30th of March 2016.

Sediment concentration in the aquaria was measured by filtering two liters of water from each aquarium on pre-weighted 48 mm Whatman GF/F glassfiber filters (Whatman Inc USA). Filters where subsequently dried for 24h at 60 ° C, incinerated for 4 hours at 400 C and weighted. The procedure was repeated twice a week.

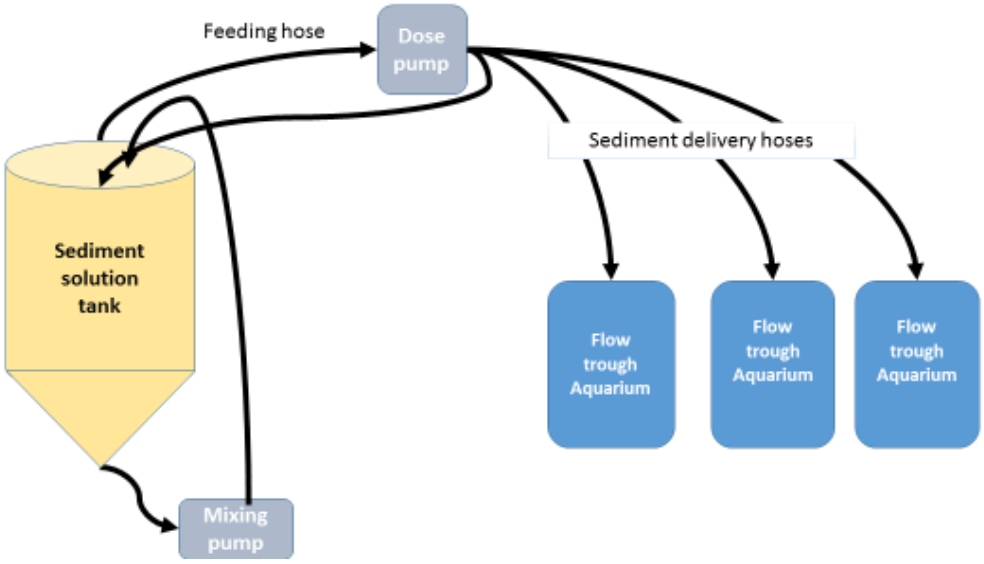

Figure 4: Schematic view of the experimental design, arrows show water flow direction

3.3.3 Coral monitoring.

Time-series pictures of each individual coral were taken every two weeks during the experimental period for monitoring of behavioral activity. Pictures were taken with same equipment mentioned in the time lapse picture section, no tripod was used.

Using the first and last picture session, polyp loss in Red tree corals was calculated. Two to three sections were chosen from each individual and identified in the first and last picture series. Polyps were counted and loss assessed.

3.3.4 Food enrichment with stable isotope ^{13}C.

The single celled diatom *Phaeodactylum tricornutum* (Bohlin, 1897) was cultivated from an anexic clone in Guillards f/2 algal culture media(Guillard & Ryther, 1962) in artificial sea water aquil media (Morel, 1975; Price et al., 1989). The cells where marked by replacing 100% of the ^{12}C bicarbonate in the artificial sea water by 99% ^{13}C Bicarbonate of 97% purity (Cambridge Isotope Laboratories, Inc, USA). The algae were reared on 24h light regime at 14.5±1.5℃ for two weeks until it reached an absorbance (optical density) of 0.328 (dimensionless) measured at 750 nm (light scatter of cell walls), measured in a Jenway 6715 UV\VIS spectrophotometer and a 1 cm cuvette. Two algal samples of 10 ml were filtrated in pre-combusted(400° C) Whatman 25mm GF\F Glass-fiber filters, then placed in an air tight container with 37% HCl acid vapor for 1h to further eliminate inorganic carbon, samples were stored at -21 ° C for 1 month, then gently dried for 24 hours at 60℃. The samples were individually packed in 5mm x 8mm silver capsules weighted and sent for Isotope Ratio Mass Spectrometry (IRMS) $^{13}C\backslash^{12}C$ ratio analysis. IRMS is a specialization of mass spectrometry that measures the relative abundance of isotopes in a sample.

Cysts of brine shrimp (*Artemia salina)* were put to hatch in hatching cones of 1.5L filled with filtered sea water at 28° C for 24 h. Cysts were added at a concentration of 1.5 g per liter After hatching the nauplii were transferred to 15 L tanks and reared for two days feeding on 400 ml of the ^{13}C marked algal suspension per day. The nauplii were then taken from the water and frozen at -21° C until used.

Two samples of the brine shrimp culture were gently dried at 60° C, grinded, and acid fumigated with 37% HCl vapor for 48 h. 1.5 mg dry weight of each sample was packed into silver capsules, and send for IRMS analysis.

Algae was cultivated in the same manner as isotope marked algae but without replacing the ^{12}C bicarbonate by ^{13}C and fed to a culture of brine shrimp. The latter was also frozen until used. Samples of the unmarked algae (no ^{13}C) and *A.salina* were also taken and treated the same way before sending them for IRMS analysis.

Corals were fed the ^{13}C marked brine shrimp twice a week during the last week of the experiment. At the culmination of the experiment all of the *D.florida* specimens from each aquaria were dried, grounded, acid fume treated for elimination of inorganic

C, and redried. 1.5 mg dry weight of each individual was packed into silver capsules and sent for IRMS analysis. *P. resedaeformis* tissue was stripped from the gorgonin skeleton before being processed in the same manner as the brine shrimp samples.

All IRMS $^{13}C\backslash^{12}C$ analyses were performed by elemental microanalysis laboratories based in the UK using a continuous flow IRMS instrument. Results were given in δ notation which is parts per thousand deviations from a standard with a known and stable ratio. The standards are distributed from the International Atomic Energy Agency in Vienna, Austria. A positive $^{13}C\,\delta$ value denoted relative enrichment of ^{13}C and negative values denotes depletion relative to standards. Meaning that the lower the $^{13}C\,\delta$ the less amount of heavy isotope is in the sample relative to the standard.
The $^{13}C\,\delta$ values were transformed into $\Delta\,\delta\,^{13}C$ using the following equation $\Delta\,\delta\,^{13}C = {}^{13}C\,\delta$ consumers- $^{13}C\,\delta$ food source. Transformation was done in order to relate and standardize the values in relation to the food source. Higher values reflected a higher amount of ^{13}C based food intake (Zanden & Rasmussen, 2001).

3.4 Histology

Prior to terminating the corals, one tissue sample of each species from MT, GB treatment and control was taken (six samples in total). The Red tree coral samples were taken by removing a small portion of a branch, containing 3 to 5 polyps. The samples were put in 100 ml sea-water with 50 gr menthol crystals for 1 hour in order to anesthetize them. Samples were fixed in 10% formalin for 48 h before being transferred to 70 % ethanol. The samples were subsequently extracted and placed into embedding and processing cassettes and left over night in a Leica TP1020 automatic tissue processor in order to paraffin infiltrate the tissue. The processes aim to dehydrate the tissue through successive baths of graded ethanol before infiltrating the tissue with molten paraffin. After this step each sample was embedded by placing it in a molt in the desired direction. The bottom part of the cassette was placed on top, and then filled with paraffin using a Leica paraffin dispenser EG1120. When cooled down, the paraffin blocks were demounted and cut into 4 µm thick sections using a Leica Jung autocut-microtome. The sections were placed in a 46 ° C water

bath then they were floated onto glass slides Two sections per samples were stained with Harris hematoxylin and eosin (H&E), and then the cover slide was glued on with tissue mount.

The slides were then observed under a Zeiss Axioskop 2 plus microscope at magnification x40 and the concurrent pictures were taken using a Nikon digital sight DS-U1 camera. When particles where found embedded in the tissue pictures were taken. 29 pictures were taken for both coral species of the slide under MT treatment. The particles found were counted and, their diameter measured using Image J 1.49 software (Abràmoff, Magalhães, & Ram, 2004).The diameter was measured by taking the longest length for each particle.

3.5 Statistical analyses

3.5.1 Polyp count

The percentage loss was calculated and analyzed using R software (R core team 2014). Because of the binomial nature of the data a Generalized Linear mixed effect Model (GLMER) with lme4 package (Bates et al., 2015) was used. The mixed effect model was to take into consideration the nested random effects of the aquaria and individuals.

3.5.2 Isotope ratio analyses

The R software was used. A linear mixed effect model (LMER) was used to analyze the data in order to account for the nested effect of the aquaria. A full model comparing the two treatments was done. A likelihood Ratio test was done to assess the significance of the treatments, as to evaluate if they had an effect on the specimens $\Delta\ \delta^{13}C$ value.

3.5.3 General

Box-and-whisker plots were used to graphically illustrate the distribution of the data, and to graphically compare the different treatments. The plot distributes the data in quartiles. Quartiles separate the original set of data into four equal parts. Each of these parts contains one-fourth of the data. A quartile is a number, it is not a range of values. A value can be described as “above” or “below” the first quartile, but a value is never “in” the first quartile. The second quartile

is the median value. When comparing different data sets if the third quartile of one group is above the second quartile of the second group it hints a significant difference between the groups, in the same manner if the second quartile of one group is below the first quartile.

4 Results

4.1 General biology

4.1.1 Activity

After observing the time-lapse pictures, no distinguishable pattern on the activity in any of the species was defined as a function of the time of the day, nor as a function of time. The coral polyps did not expand at any specific time of the day.

Duva florida specimens open and closed with no regular pattern, and intermediate states where the coral was not fully open nor closed were also noted (Fig: 6).

Primnoa resedaeformis individuals didn't show any pattern in their polyp activity. They were open, contracted or in an intermediate state (Fig. 7).

Before starting the sedimentation experiment temperature in the aquaria ranged from 9.2 to 7.9 ° C. During the period where temperatures where above 8.7 in 3 of the aquaria individuals looked ill and were dying. Ten *D. florida*, and nine *P. resedaeformis* individuals died. The remaining Cauliflower corals were redistributed between the 9 aquaria to achieve 2 to 3 individuals in each. The Red tree coral individuals where replaced with fragments of the colonies that where left in the acclimation tank

4.1.2 Feeding behavior.

Cauliflower corals capture their food item with their tentacles and drag it to the mouth cavity and it was possible to observe the nauplii inside the mouth (Fig.8A). A sticky mucus net approach was also observed (Fig. 8B).

Red tree corals released a sticky mucus thread used as a "food capturing net" where the food items got trapped, similar to "sticking cells" of Ctenophores. The net was then dragged towards the polyp and most likely ingested (Fig. 9). The polyps from where the net was observed where not completely opened.

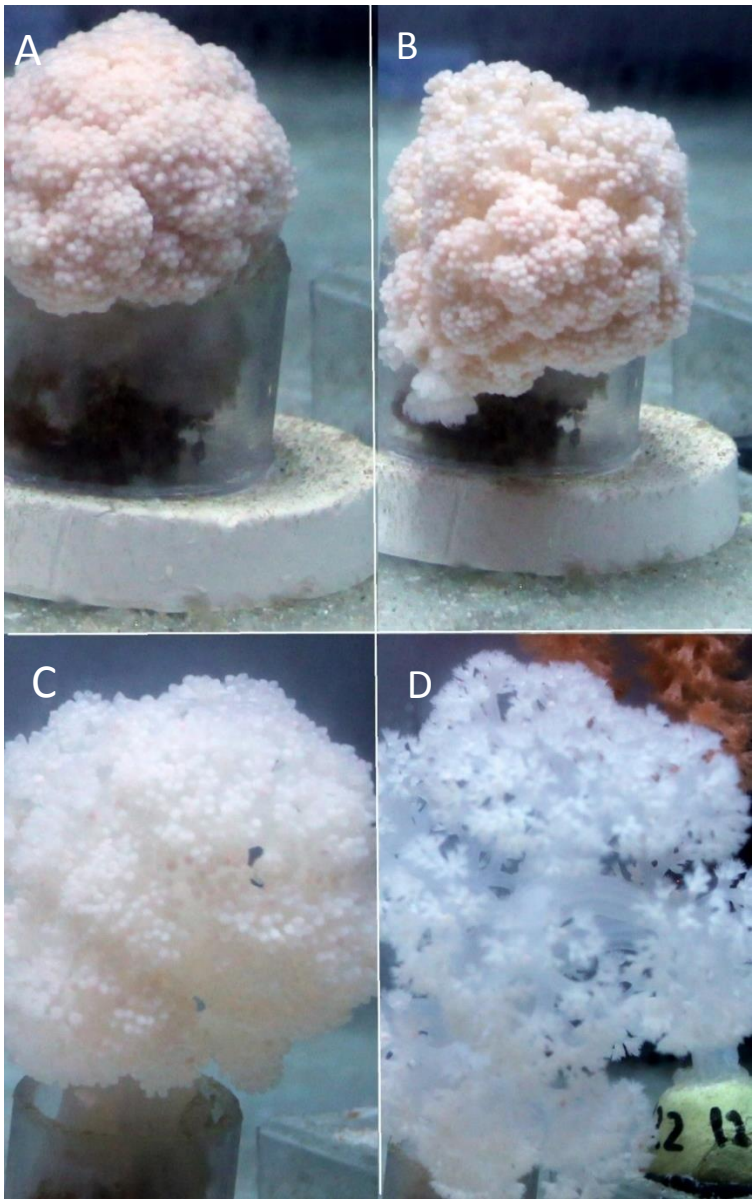

Figure 5: Different expansion stages of the same individual of Cauliflower. A: The specimen is completely contracted; the individual polyps can be still distinguished. B: The coral is starting to expand, the inner parts first. C: expansion continues, the branches are almost completely out, but the polyps are still contracted. D: The coral is completely expanded. The polyps and the tentacles can be observed. Photo collage done using the first time lapse picture series.

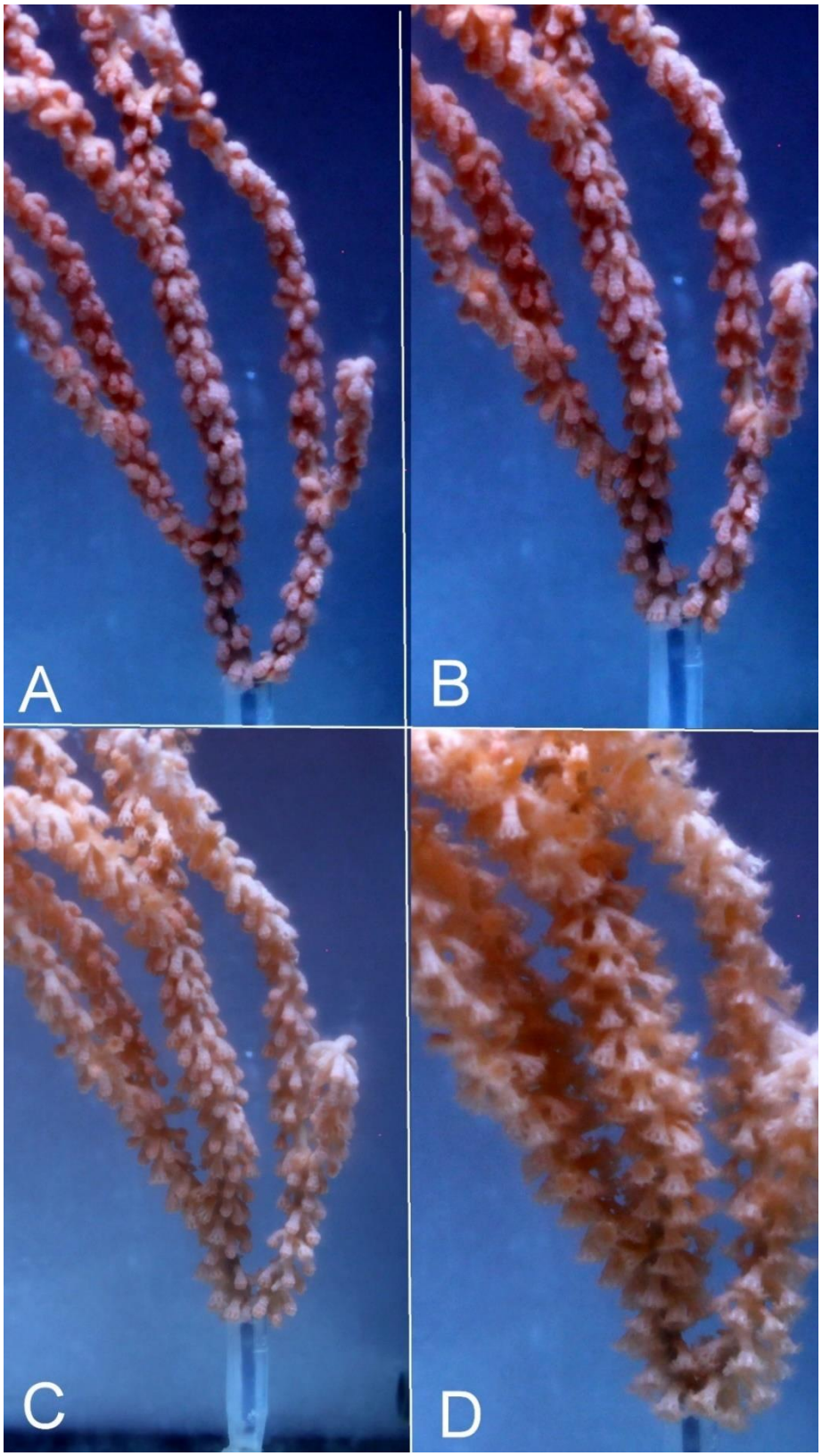

Figure 7: Different degree of polyp expansion of one specimen of Red tree coral

A: Polyps are contracted and close against the gorgonin branch/skeleton.

B: Polyps beginning to expand.

C: 50% expanded polyps.

D: completely expanded polyps, the tentacles can be observed, and the coral has an overall "bushier" appearance.

Collage done using the pictures from the second time lapse series.

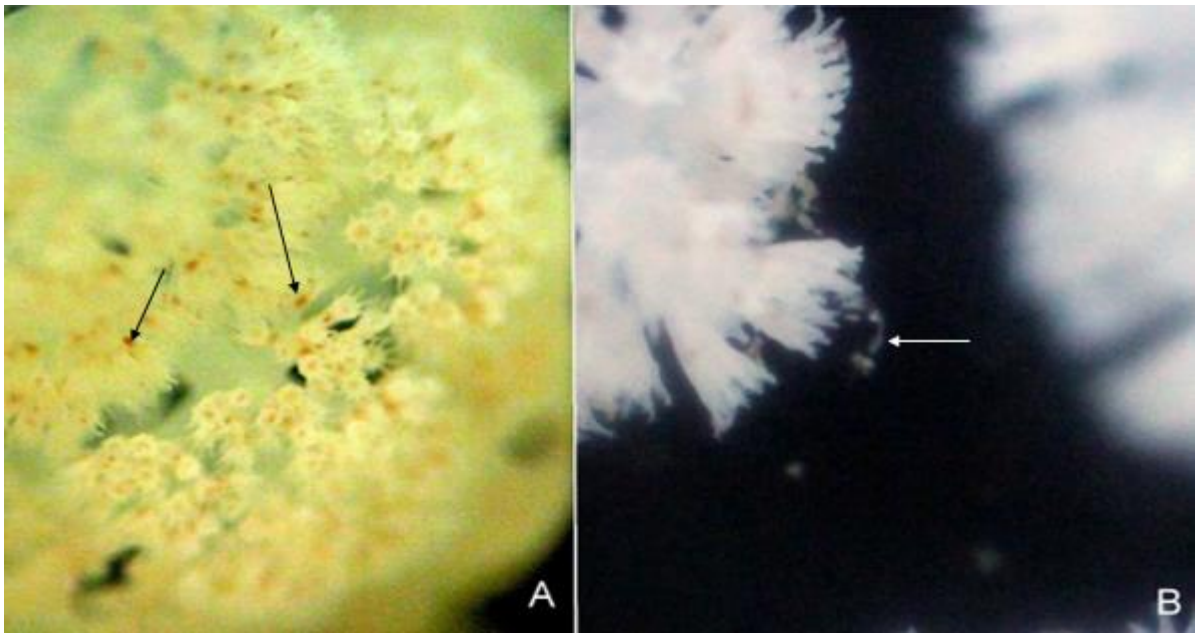

Figure 8: Feeding of *Duva florida*. A: The *Artemia salina* nauplii can be observed as a redish dot inside the individual polyps, caught using the tentacles to sting and capture the prey (black arrows pointing to the nauplii inside the polyp). B: Some polyps released a mucus tread where prey was captured, white arrow pointing to the small mucus tread.

Figure 9: Feeding of *Primnoa resedaeformis*. Some polyps release a mucus tread that acted as a sticky fishing net trapping the zooplankton. The net was eventually dragged to the polyp.

4.2 Sedimentation experiment.

Particle distribution of the mine tailings (MT) and the glass beads (GB) was not the same. 60 % of MT particles size were between 1-20µm diameter. While 15,6 % of GB were between 1-20- m see (Fig. 10 and 11). The difference in shape of the particles is illustrated in Fig. 12.

Mean sediment concentration measured trough filtration for MT treatment was 18.79 mg L^{-1} (Standard deviation SD= 8.65), and for GB 17.74 mg L^{-1} (SD = 7.03). For the control group, mean sedimentation was 10.57 mg L^{-1} (SD = 2.21). For the concentration in each aquarium see Fig. 13.

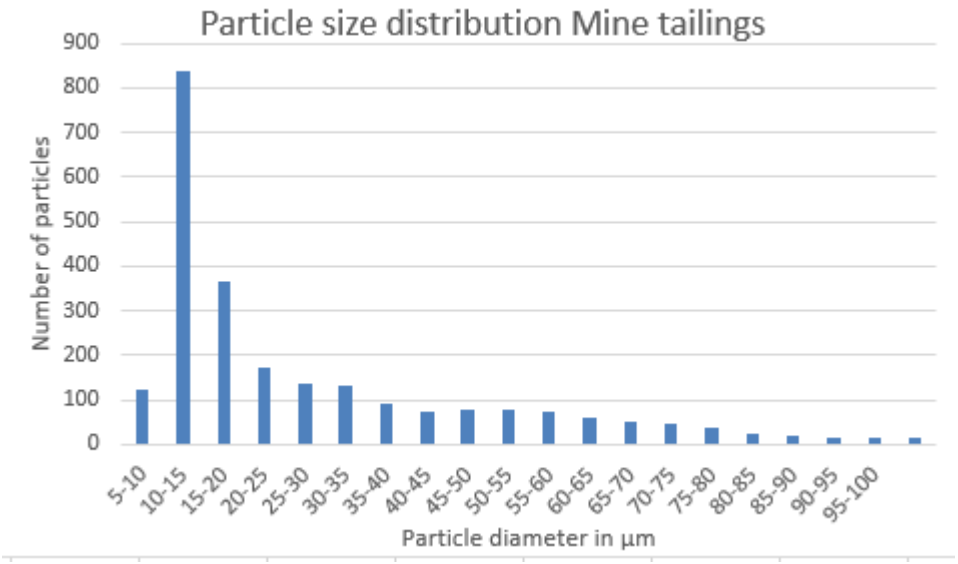

Figure 10: Particle size distribution of mine tailings, majority of the particles are in the size class 10 to 25 µm.

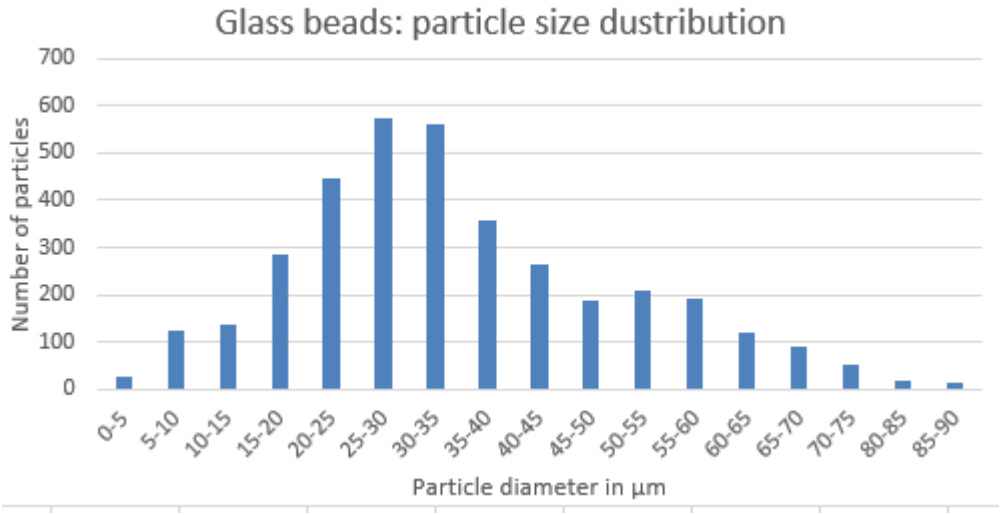

Figure 11: Particle size distribution of GB, majority of particles are in the 20 to 40 µm size range.

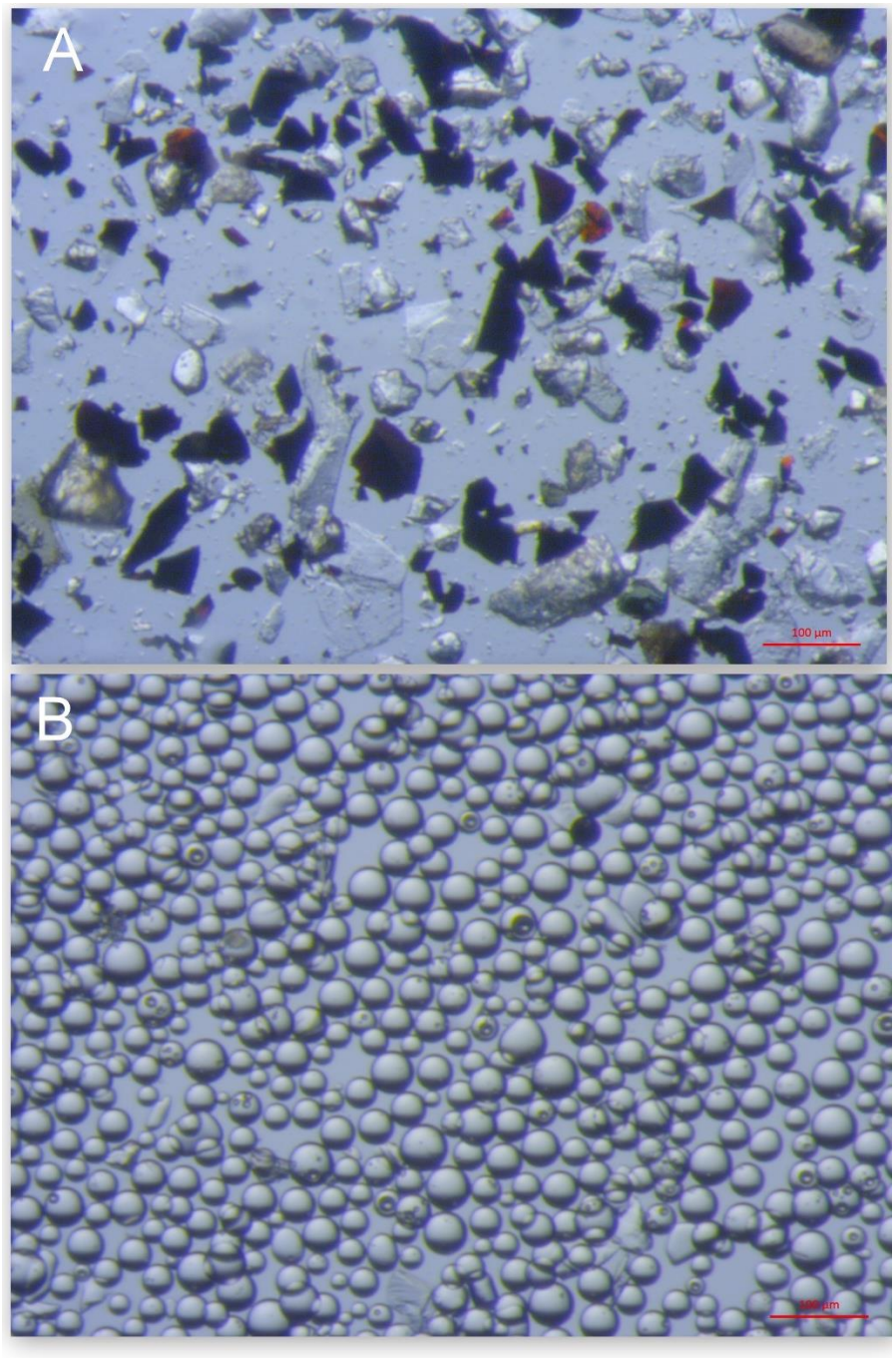

Figure 12: Particles seen under the stereoscope. A: Mine tailings (MT) particles. B: Glass beads (GB) particles. The scale bar on the left low corner represents 100µm.

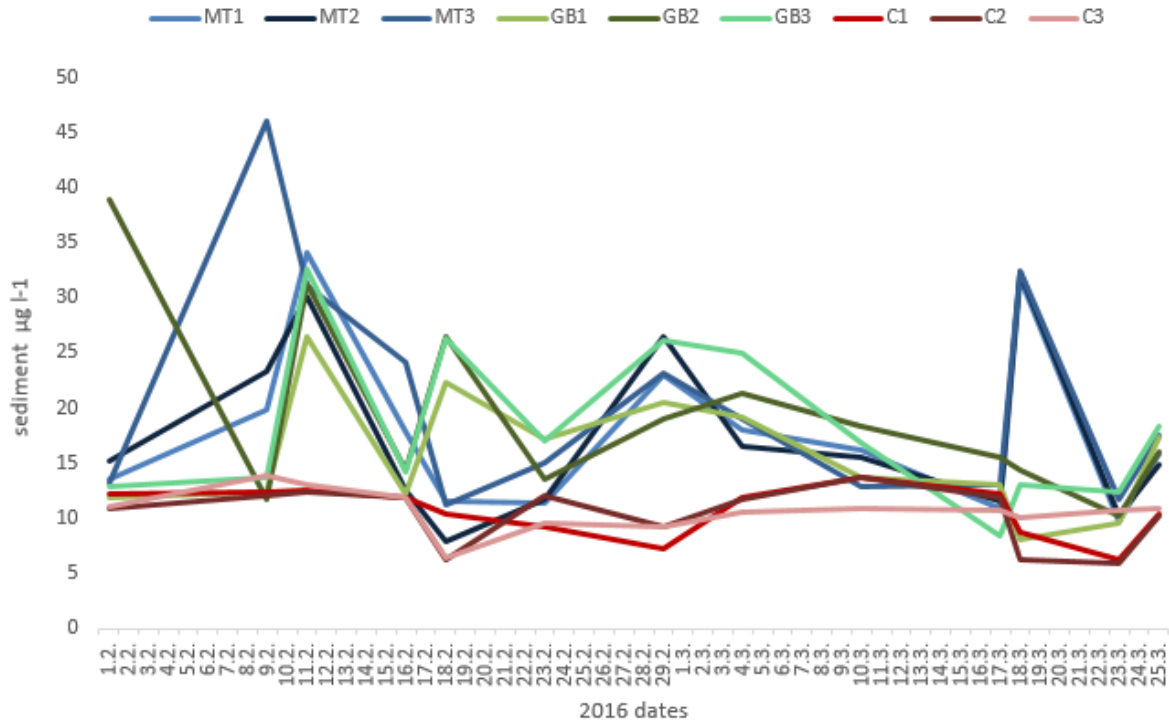

Figure 13: Sediment concentration in the water for each aquarium throughout the experiment mine tailings (MT) aquaria. Glass beads (GB) aquaria. Control (C) aquaria. Number denotes the number of the aquaria.

4.2.1 *Duva florida*

Individuals exposed to MT treatment were contracted when the sedimentation cycle was on, and turbidity in the water was still visible after cycle stop. Nevertheless, by the end of the 6 hour pause the corals were expanded again. Mine tailings accumulation was observed on top of some of the contracted individuals (Fig. 14A).

Specimens exposed to GB treatment didn't show any physical signs of stress or contraction during the treatment period (Fig.15). Control individuals did not show any signs of change (Fig. 16).

In the next three pages the figures show the progression of the Cauliflower coral trough the different treatments.

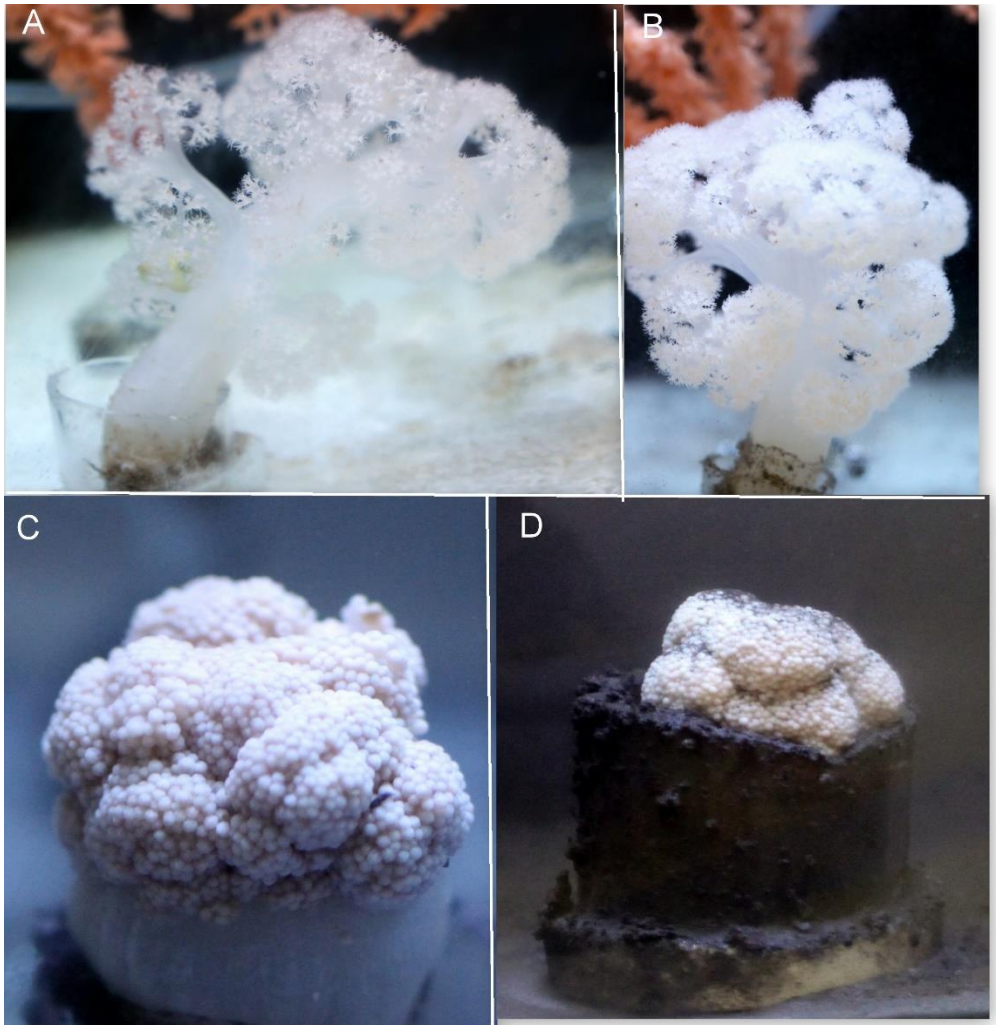

Figure 14: Cauliflower coral Individual exposed to mine tailings. A: first picture before starting the experiment (12th of January). B: Second picture (27th of January). C: Third picture (17th of February). D: Fourth picture (21st of March). In this picture the coral was alive and retained its physical integrity, but it was observed mostly contracted and with sediments on top.

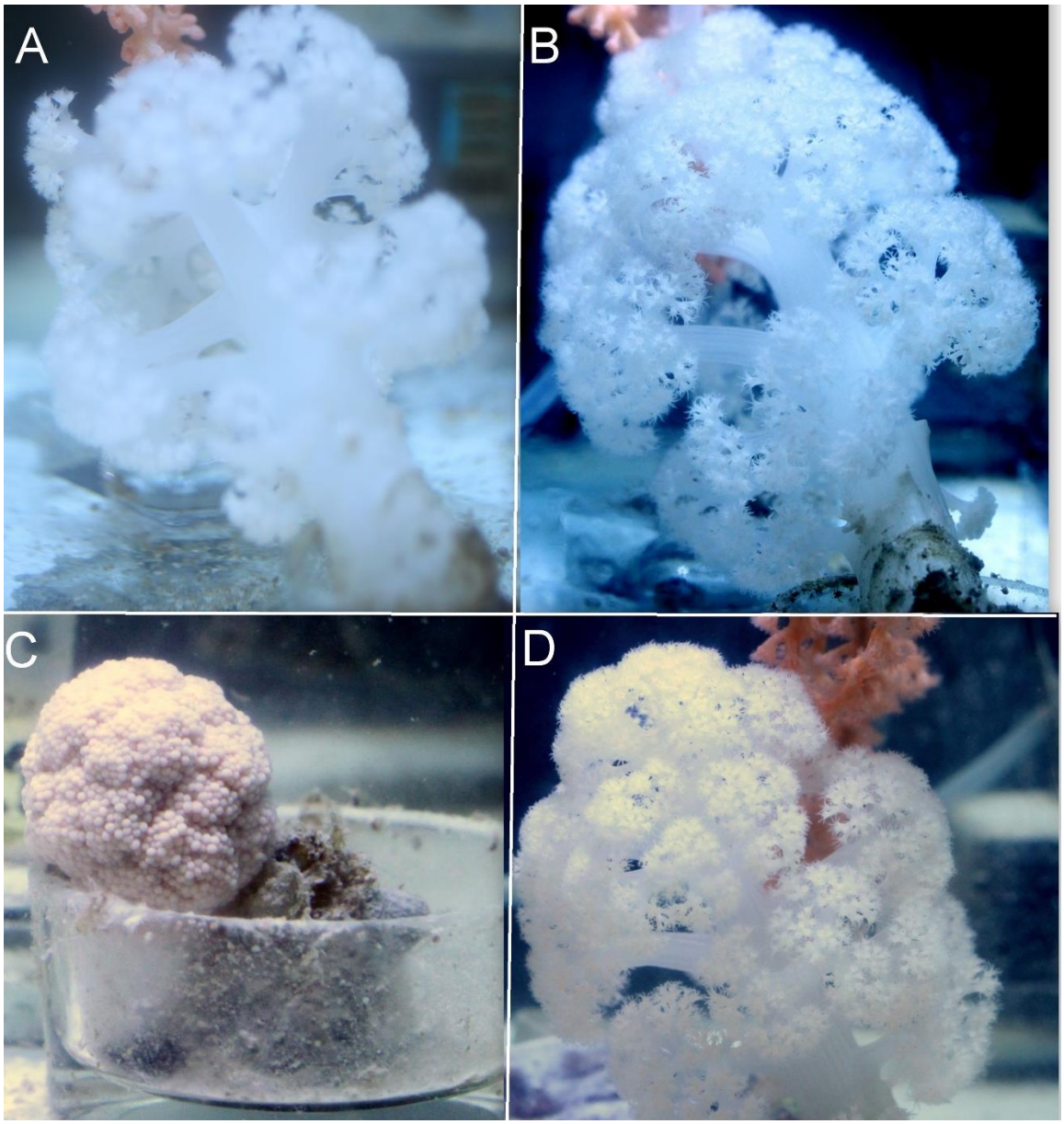

Figure 15: Cauliflower coral individual exposed to GB sedimentation. A: 12th of January, before starting the experiments. B: Picture taken the 27th of January. C: 17th of February. Particles can be seen accumulating on the plastic holdfast. D: 21st of March.

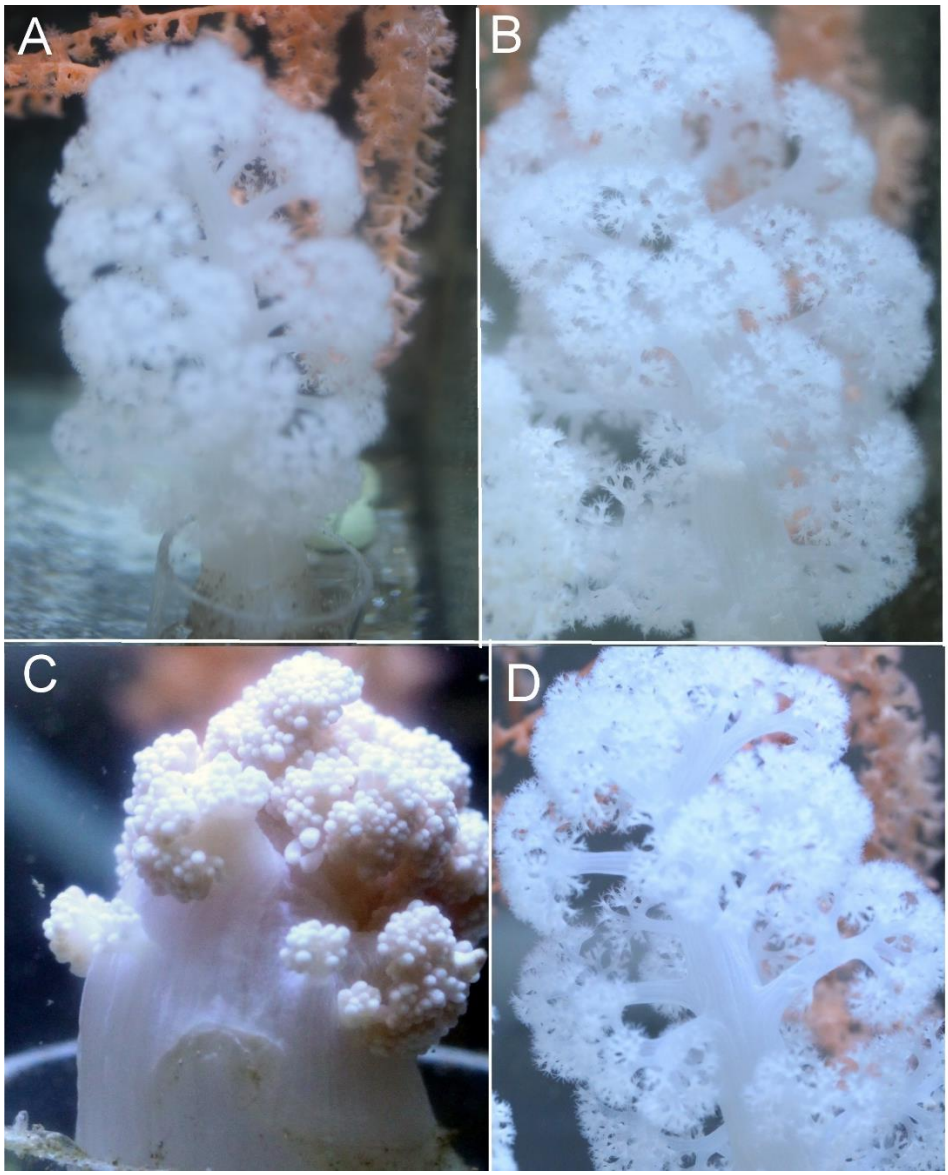

Figure 16: Cauliflower coral control individual throughout the experiment duration. A:12th of January. B: 27th of January. C: 17th of February. D: 21st of March.

4.2.2 *Primnoa resedaeformis*

In general polyp loss was first observable in the tip of the branches.

Individuals under MT exhibited polyp loss. They lost 34 % (SD = 13) of their polyps in the areas counted (Fig. 17). Sediment accumulation on certain areas could be seen enveloped in mucus (Fig. 20).

GB treatment subjects also exhibited of polyp decline (Fig. 18). They lost 38 % (SD = 12).

Control specimens also had polyp loss, but to a lesser degree than the individuals under treatment with 19 %(SD = 21) loss. The large SD in due to one control aquaria that had a polyp loss of 32% (SD= 26). This is included in the general average for control individuals.

The polyp loss difference between Control group and GB treatment was significant (p-value = 0.014). Also the difference between control and MT was significant (p-value = 0.0330) (Fig.21A).

The same test was done excluding the control aquaria with a hit polyp loss, the results where similar. Average percentage loss in control individuals was then 14% (SD = 15) in GB 38 % (SD = 12) and MT 34 %.(SD = 13) The difference in polyp loss was also significant (p-value <0,01 in both cases) (Fig. 21B).

The following pages contain figures illustrating some of the reactions of Red tree coral to the experiment.

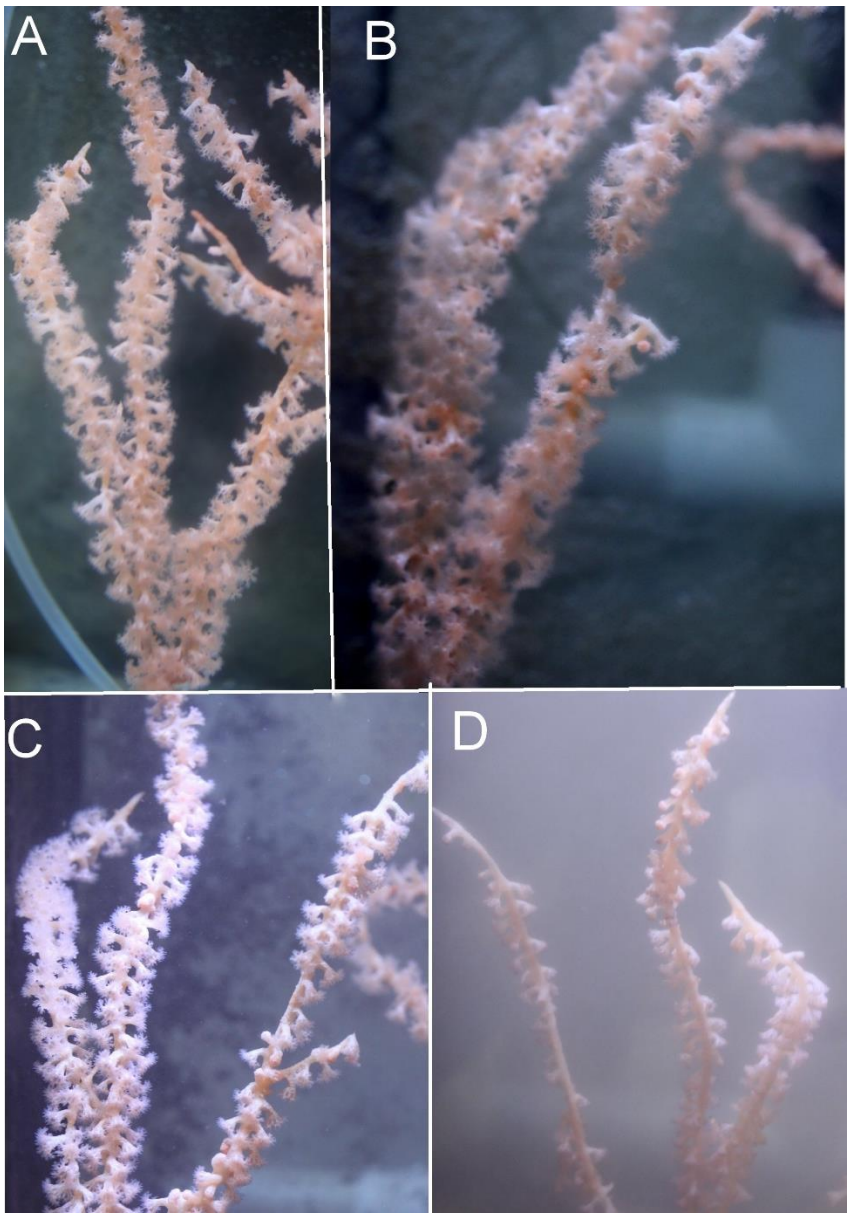

Figure 17: Red tree coral individual under MT sediment load experiment. A: 12th of January, before starting the experiments. B: Picture from the 27th of January. C: 17th of February. D: 21st of March, the polyp loss is noticeable.

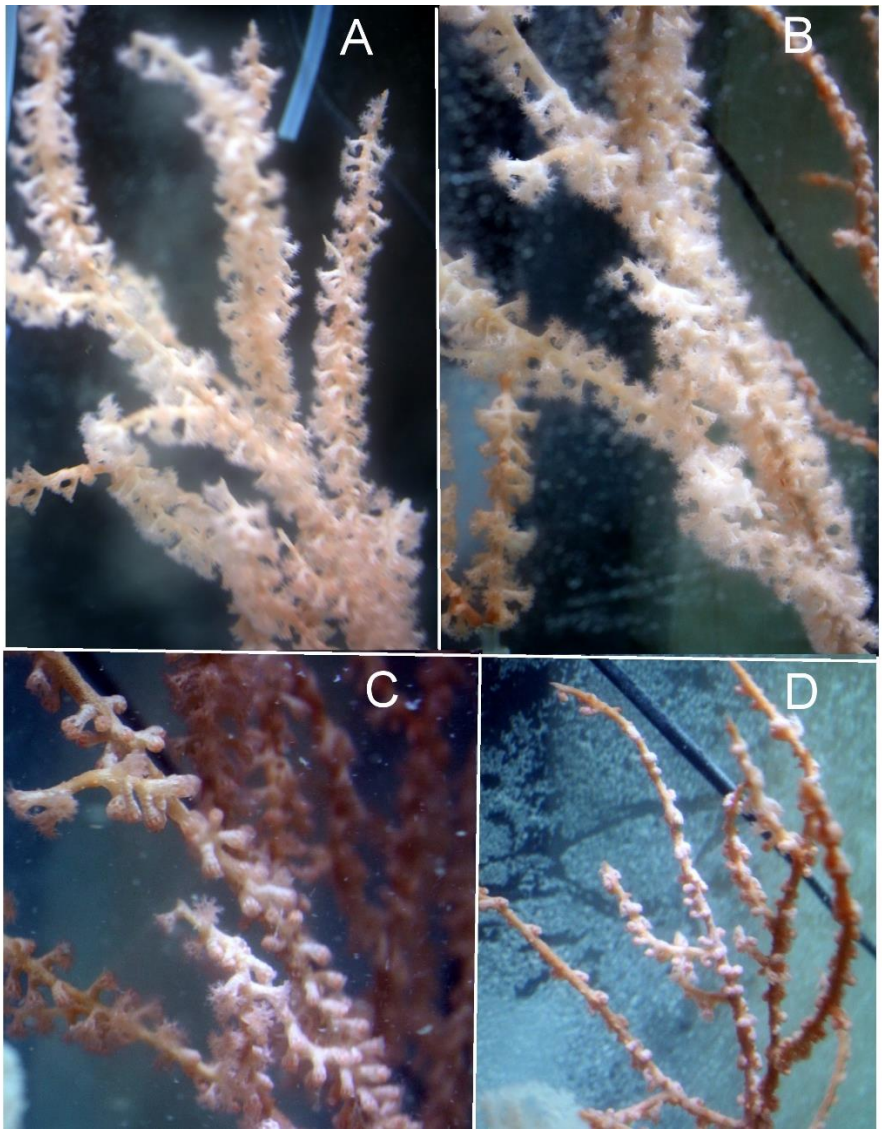

Figure 18: Red tree coral under GB treatment. A: 12th of January, before starting the experiments. B: Picture taken the 27th of January. C: 17th of February. D: 21st of March .When comparing picture A and D polyp loss can be observed even if the polyps in picture D are contracted.

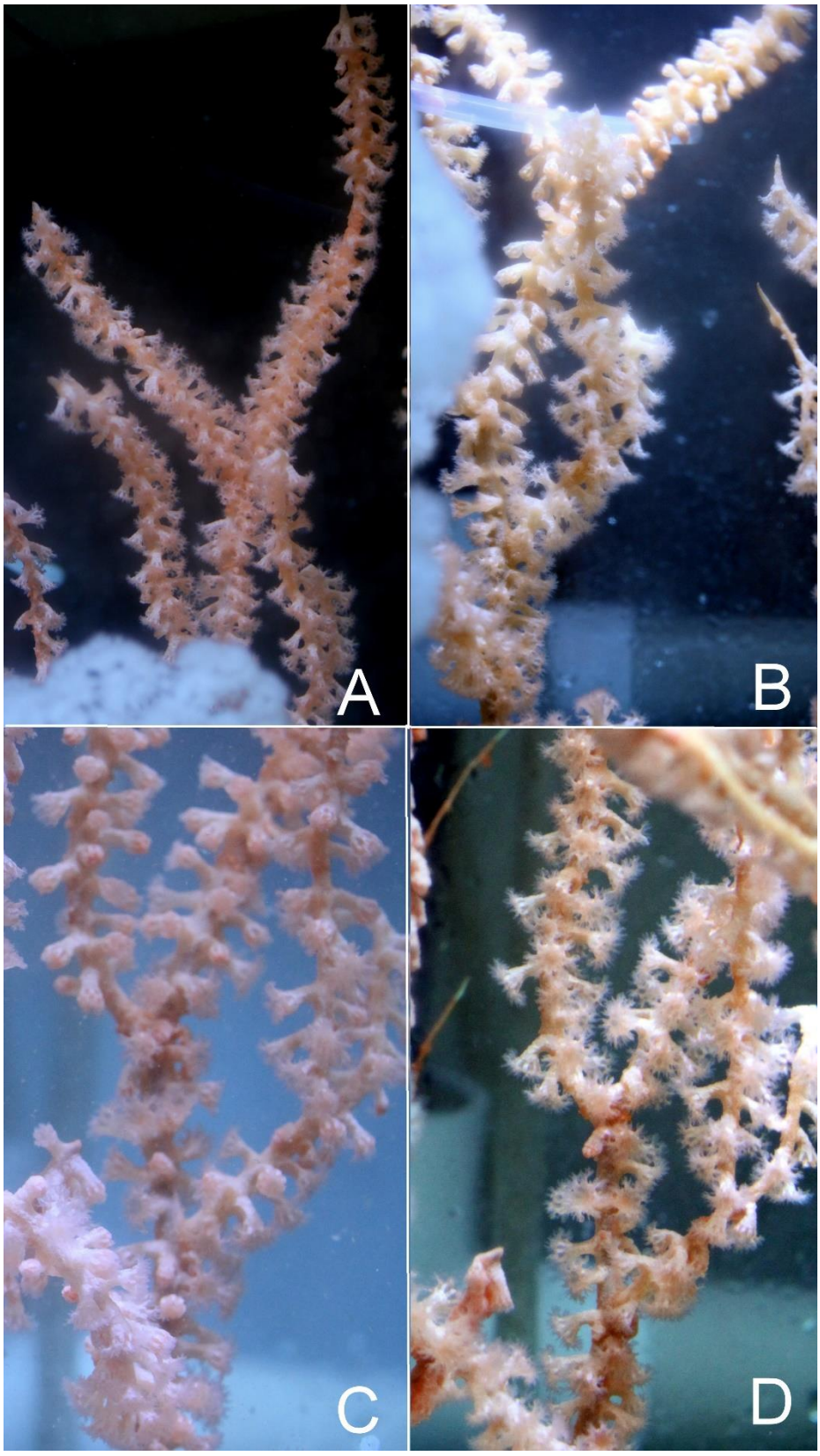

Figure 19: Red tree coral control individual A: 12th of January, before starting the experiments. B: Picture taken the 27th of January. C: 17th of February. D: 21st of March.

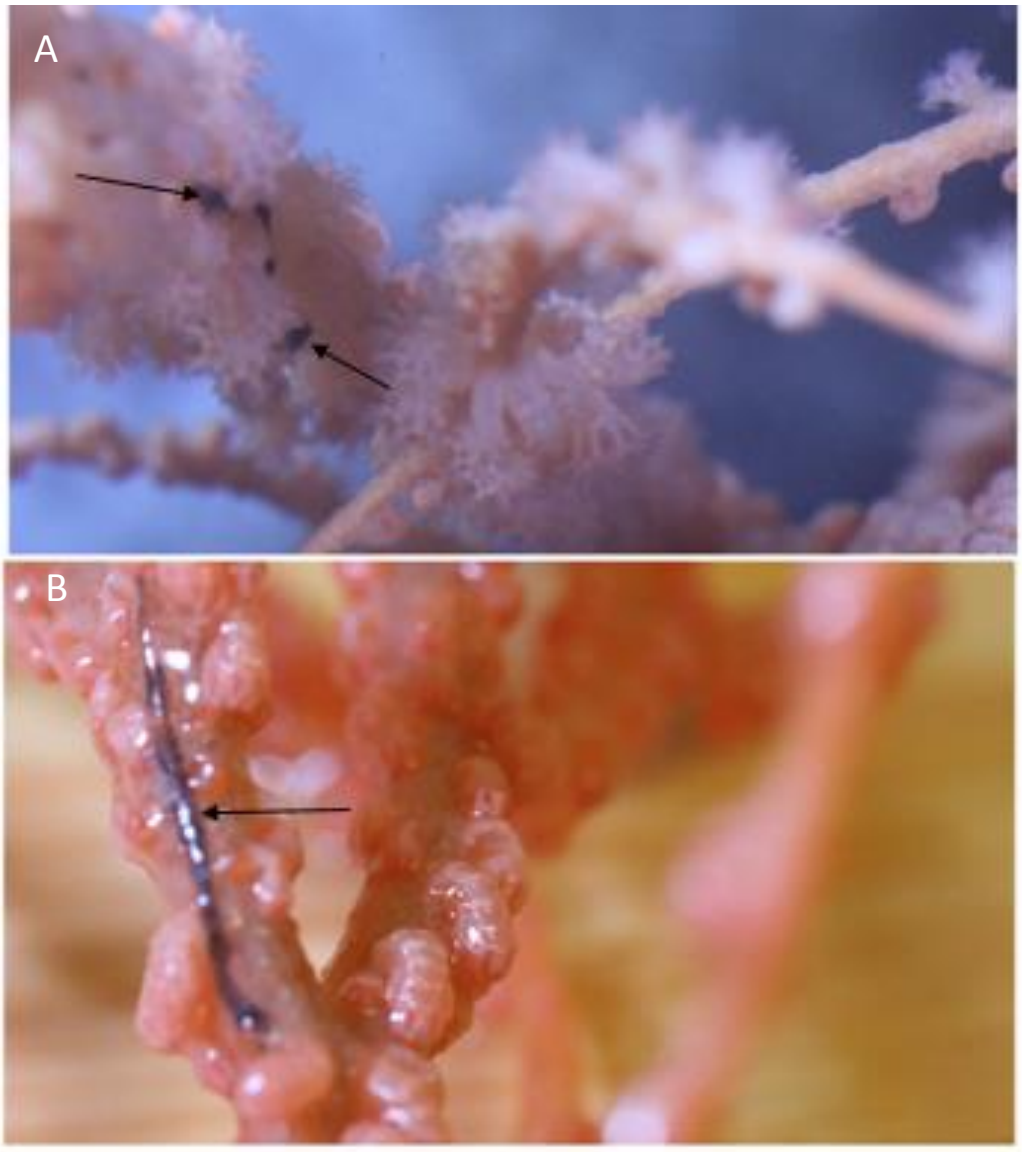

Figure 20: Mine tailings accumulation in Red tree coral captured in a mucus layer. Arrows pointing to the encapsulated MT. A: Mucus observed in the coral while being in the aquarium. B: Mucus observed in a coral outside of the water before killing it. Here the stickiness of the mucus can be observed.

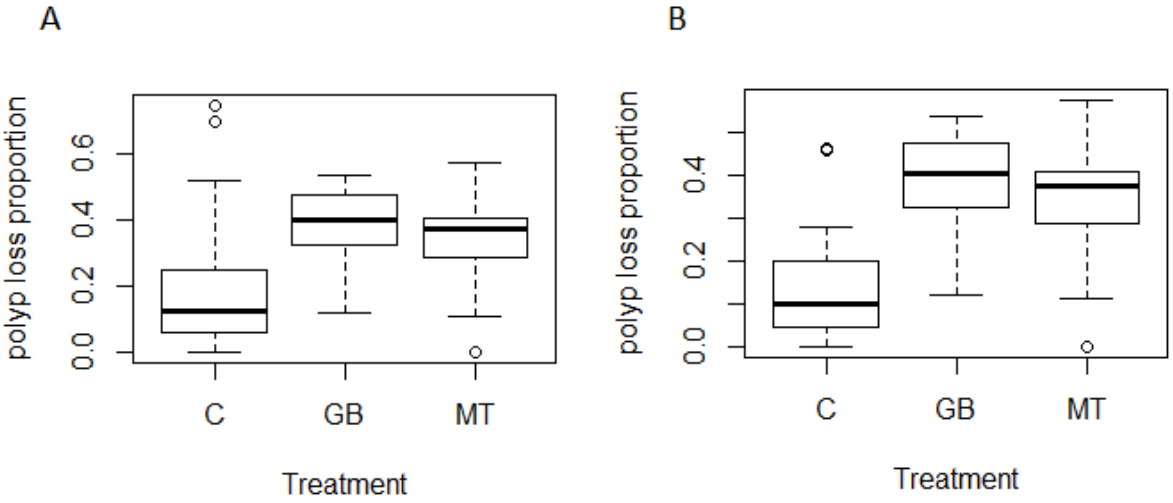

Figure 21: Box-and-whiskers plot representing polyp loss proportion. of Red tree coral A Including all data set, the median (represented by the thick bar) from MT and GB is above the third quartile of the control group, which suggests that the polyp loss is statistically significant relative to control (C). B: Data set excluding the control aquarium where 32% polyp decline was observed. The trend is the same as in the complete data set. The whiskers represent length the range of the data, its dispersion.

4.3 Food intake in corals $^{13}C\backslash^{12}C$ ratio

The reared algae had such a high amount of 13 C that the IRMS was saturated. The enriched nauplii had a 13C δ value of 529 ‰ while the 13C δ of non-enriched nauplii was -19,67‰.

4.3.1.1 Duva florida

Higher Δ $\delta^{13}C$ values means a higher amount of ^{13}C thus a higher food intake. Control individuals had a mean Δ $\delta^{13}C$ value of -535.820 ‰ (SD = 6.04). MT treated individuals had a mean Δ $\delta^{13}C$ value of -546.003 ‰ (SD = 3.96) while GB specimens had mean Δ $\delta^{13}C$ value of -542.414 ‰ (SD = 8.48). The Δ $\delta^{13}C$ values indicated that Control individuals ate more than GB and MT treated specimens, and MT treated individuals ate less than GB treated ones. The box plot figure 22 indicated that the treatments were statistically significantly compared to the control which is for the MT treatment.

Likelihood ratio test showed that MT treatment had a significant effect on the Δ $\delta^{13}C$ values when compared to control. (p-value=0.001844) which is diminished on average by -10.186 ‰. GB treatment did not reveal any statistically significance

difference in the Δ δ^{13}C values when compared to Control (p-value= 0.07719) though the trend is a decrease, on average -6.59 ‰.

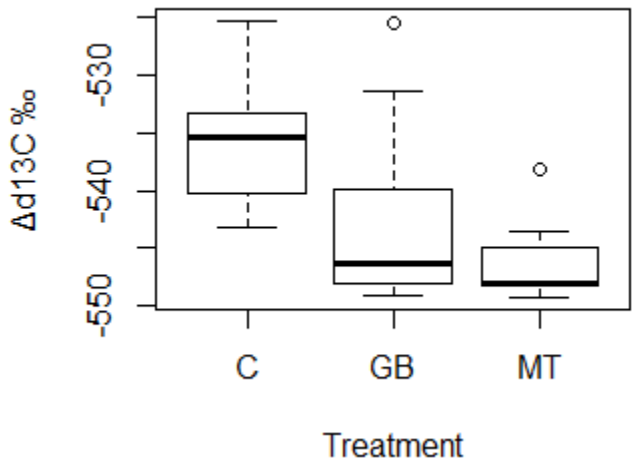

Figure 22: Boxplot Δ δ^{13}C‰ in *D. florida.* A clear pattern emerges; the Δ δ^{13}C values for the Control individuals are higher than for GB treatment individuals, which are higher than the values for specimens under MT. The median for GB and MT are lower than the first quartile in the C groups, this indicates that GB and MT Δ δ^{13}C‰ are statistically significantly lower than in control.

4.3.1.2 Primnoa resedaeformis

Control had a mean Δ δ^{13}C =- 542.073‰ (SD=3.04), MT treatment had a mean Δ δ^{13}C =-537.426‰ (SD=3.54) and finally GB treatment had a mean Δ δ^{13}C =-539.283‰ (SD=3.474). The results suggest that the Control individuals ate less than individuals in MT which ate less than GB individuals. The boxplot figure 23 suggested a statistically significant difference between GB and Control and MT and Control.

Likelihood ratio analysis test revealed a statistically significant increase in Δ δ^{13}C from Control to MT (4.65 ‰) (p-value = 0.008603). The increase in Δ δ^{13}C from Control to GB (2.79‰) was not statistically significant (p-value = 0.06864).

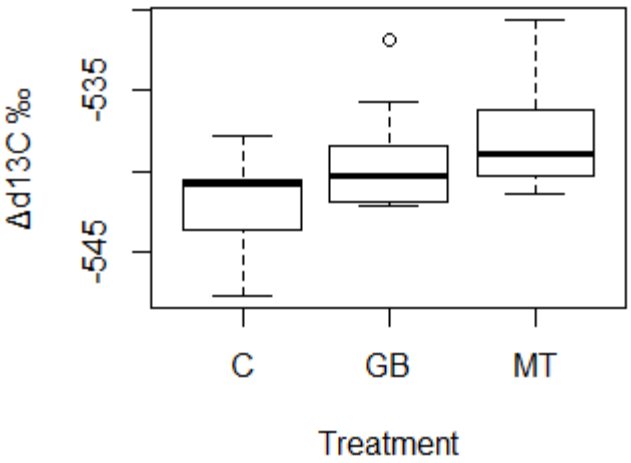

Figure 23: Boxplot of Δ δ^{13}C‰ in *P. resedaeformis*. The boxplot suggests that the Δ δ^{13}C values for GB treatment group and MT treatment group are statistically significantly different from the C group.

4.4 Histology

Histological samples of both species from MT treatment showed particles embedded in the tissue. No GB particles where observed in the histological samples from corals exposed to GB treatment.

4.4.1 Duva florida

In the 29 pictures studied 439 particles were found, their mean size was 3.97µm diameter (SD=2.58). The Particles were embedded in the tentacles epidermis, the mesoglea and some were also found deeper into the polyp (Fig. 24).

4.4.2 Primnoa resedaeformis

In the 29 analyzed pictures, 283 particles were found, their mean size was 3.43µm in diameter (SD=2.68). Particles were observed close to muscle tissue, in the tentacles epidermis, and in the mesoglea (Fig. 25)

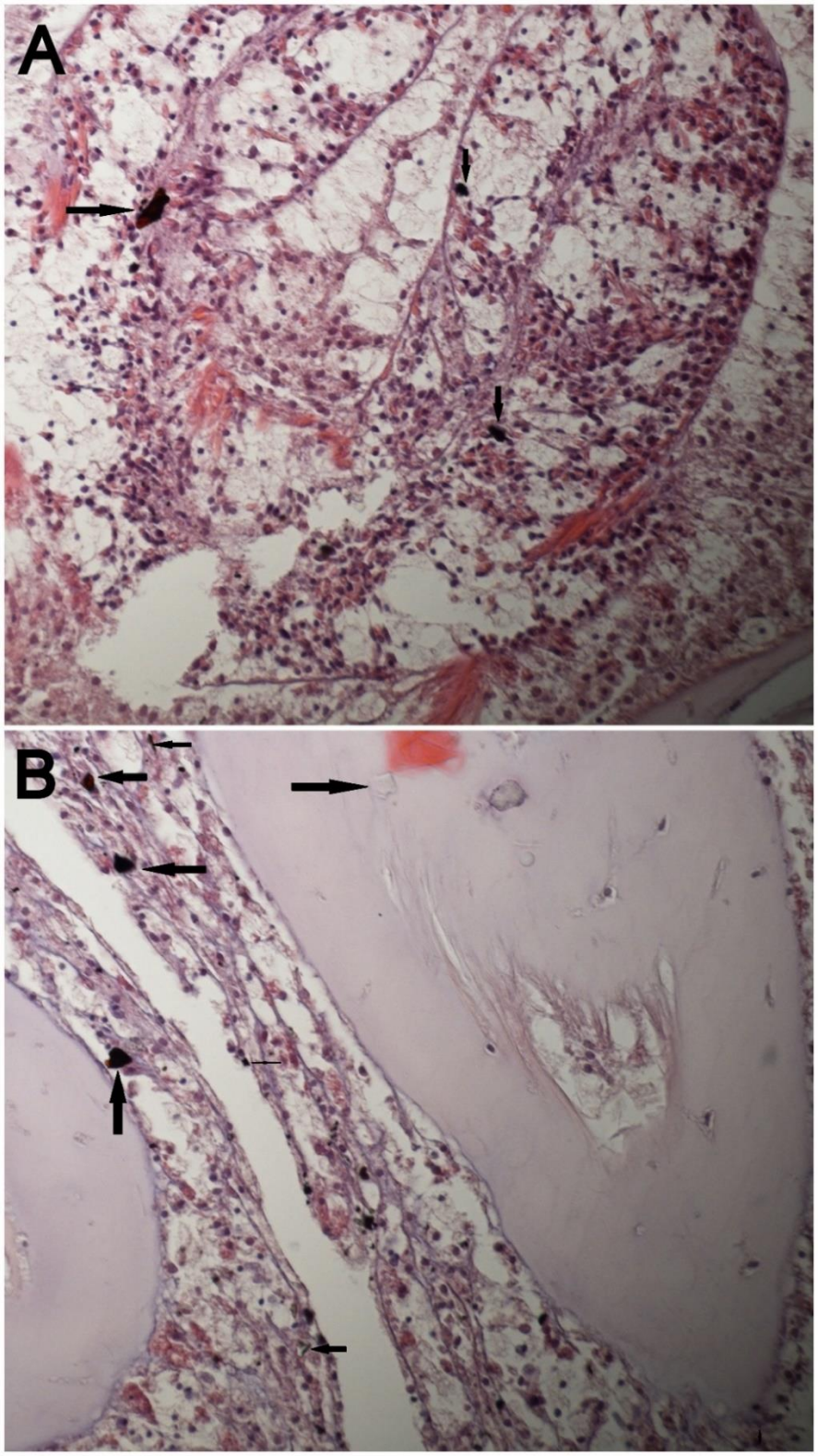

Figure 24: Histological cuts of tissue samples from Cauliflower corals exposed to MT treatment. The arrows point to the particles embedded in the tissue. A: particles embedded in the contracted tentacle. B: Particles embedded in the epidermis and in the mesoglea (close to the orange spot). Notice that the seen particles are similar to Fig. 12A

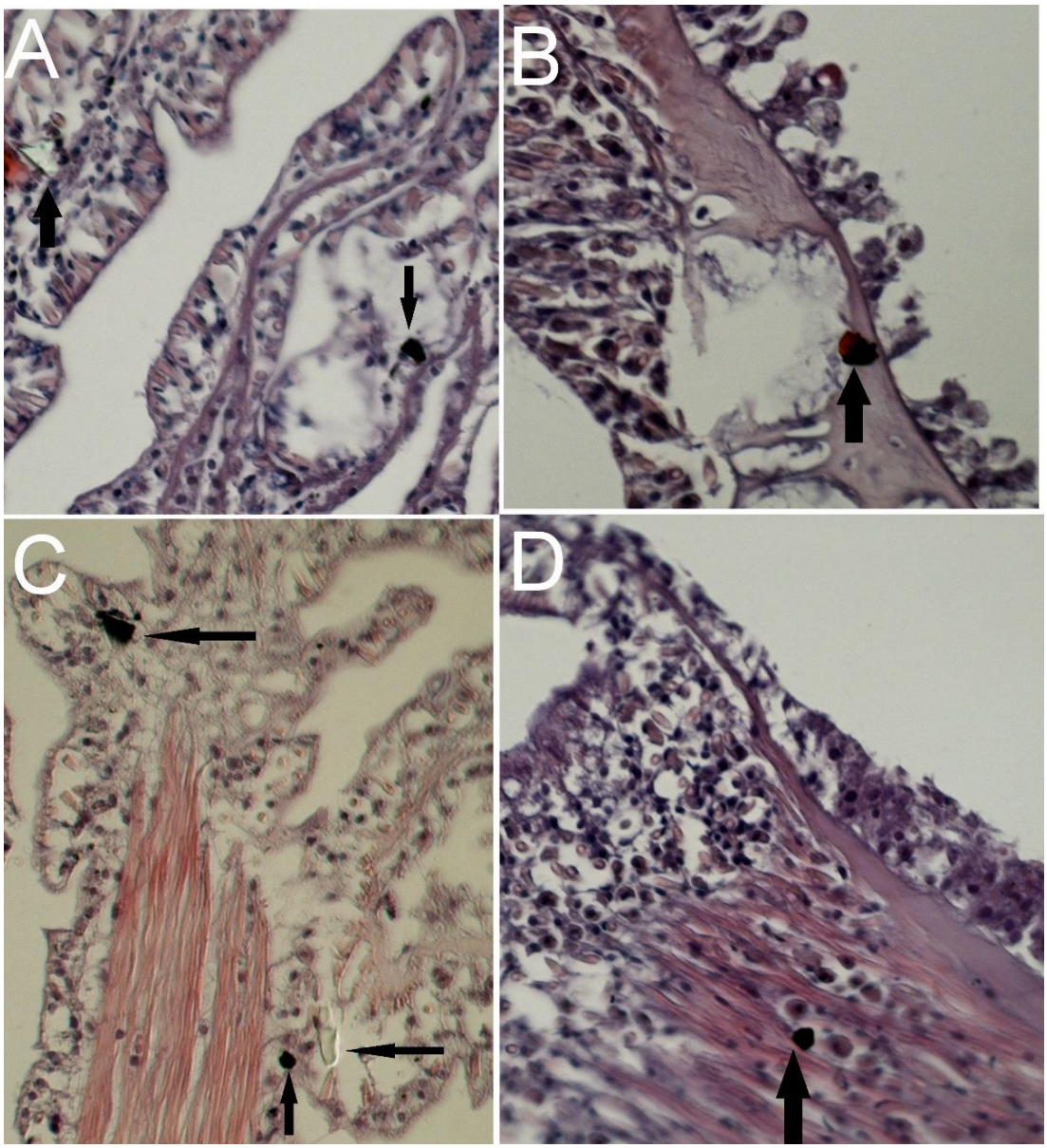

Figure 25: Histological cuts of tissue samples from Red tree coral after MT treatment. Arrows point to embedded sediment particles. A: particles embedded in the tentacles epidermis. B: Particle embedded in the mesoglea. C: particles embedded close to muscle fibers. D: particle embedded inside the muscle tissue. Notice that the seen particles are similar to Fig. 12A.

To summarize the results: The Cauliflower corals consumed less food under both treatments but only the MT treatment showed a significant decrease in food intake. The Red tree coral individuals lost a significant amount of polyps under both treatments, when compared to control individuals. Individuals in both treatments consumed more food than control individuals, but only the MT treatment was significant. Histological samples showed MT particles embedded in the tissue of both species.

5 Discussion

This study aimed to answer the following questions:

1. Cold-water soft corals respond to realistic exposure of mine tailings with respect to tissue abrasion and metabolism
    - Cold-water soft corals did respond to sedimentation of MT by changing their food intake evidencing stress.
2. Different species of cold-water soft corals responds differently to realistic exposure of mine tailings.
    - The two studied species did respond differently to sedimentation stress, the Cauliflower coral reduced its food intake, and change its behavior. The Red tree coral increased its food intake and exhibited polyp loss.
3. Cold-water soft corals respond differently to mine tailings and sediment with spherical shape with respect to tissue abrasion and metabolism.
    - The two soft coral species studied responded in the same manner to both type of sediments, nevertheless the response from MT sedimentation treatment was more marked. Also only MT particles were found embedded on the tissue of both species which might give raise to a more marked response.

5.1 General biology

The absence of marked diurnal patterns in the activity of the two coral species is a new discovery, but this can be an artifact of the laboratory conditions and the corals might react to diurnal cues in nature, such as sun, and moon light. The feeding strategies observed for *D. florida* are consistent with its presumed diet of degraded particulate organic matter (POM) as elucidated by Sherwood, Jamieson, Edinger, and Wareham (2008). The fact that they possess long slim tentacles and pinnules as seen in figure 8 (Appendix 1A for detailed morphology of Cauliflower coral polyp) support the fact that they can feed on small particles. The feeding strategy of *P. resedaesformis* is also consistent with its diet: microzooplankton and fresh POM as mentioned by Sherwood et al. (2008). Only feeding with a mucus net was observed (Fig. 9), feeding directly with tentacles using nematocysts was

difficult to spot because of polyp coloration matching the food source (*Artemia salina*). (Appendix 1B for polyp morphology).

Corals being sensitive to sustained higher water temperatures (Appendices 2 A and B) can have a synergetic effect for the corals to withstand other stressors as already pointed out by Ramirez-Llodra et al. (2011)

5.2 Reaction to sediments with different morphology

According to the experiments carried out the two coral species react differently to sedimentation of MT and GB. MT particles having a significant harmful effect, compared to GB. Different types of particles have already been proven to cause different reactions in one species of tropical scleractinian (Weber, Lott, & Fabricius, 2006) and *L. pertusa* (Larsson, van Oevelen, Purser, & Thomsen, 2013)

5.2.1 Duva florida

The Cauliflower coral specimens were mostly contracted during exposure to MT particles (Fig. 14). This behavioral change limited the time for feeding, as reflected in the $\Delta\ \delta^{13}C$ results (Fig. 22), which was statistically significantly lower than control. Reduced feeding time skew the energetic budget of the corals and diminish the growth rate as shown for *L. pertusa* (Larsson et al., 2013), and tropical corals (Anthony, Connolly, & Willis, 2002; Rolf P.M Bak, 1978; Dikou, 2009). If continued for prolonged periods it may lead to starvation. Specimens in GB treatment also showed a decreased food intake, although not statistically significantly different from control, even if the polyps were observed to be expanded also under sedimentation. This might be a sign of stress, though not as severe as stress induced by MT particles.

GB particles were not observed accumulating on top of the Cauliflower corals, which suggests that round edged particles were more easily removed compared to MT particles (Fig. 14 and 15). The finding that only MT particles were embedded in the tissue of both species supports the theory that sharp particles are more damaging to the corals (Gray, 1974). Especially the smallest fraction from 0 to 10µm which was the fraction found embedded in the tissue in the histological samples (Fig. 25). Although this has to be taken with caution since the 0 to 10 µm fraction was under represented in the GB particle size distribution. Tissue puncturing might leave the corals vulnerable to bacterial attack and subsequent tissue necrosis. Increased bacterial disease have already been correlated to increased sedimentation in tropical corals (Pollock et al., 2014)

In the case of higher sedimentation rates, more particles would accumulate on top of the contracted coral making it more difficult to reject the sediment by mucus production (Bak & Elgershuizen, 1976). A high concentration of particles at surface of coral may cause anoxic conditions and subsequent sulfides which are known to be harmful (Bagarinao, 1992).

Duva florida being an internal brooder (Sun, Hamel, & Mercier, 2011) might experience reproduction problems when being contracted for prolonged periods might also affect the planulae development, and its release. In their experiments Sun, Hamel, and Mercier (2010) showed that *Drifa glomerata* and *Drifa* sp released planulae while being fully extended. In the same study, the authors found that *Drifa* spp planulae preferred settling on hard substrata covered in biofilms which is consistent with the natural environments where nephtheidae corals are found (see appendix 3 A): clusters of hard substrate. As observed with the ROV when collecting the Cauliflower corals, they were found in *L. pertusa* gravel. Mine tailings smothering hard substrate surfaces may represent a problem for the settlement of planulae larvae.

5.2.2 *Primnoa resedaeformis*

Tissue abrasion in the Red tree coral, due to polyp loss, was more marked on individuals under GB treatment though it was not statistically significantly different from MT individuals, perhaps because the particle size distribution was bigger than the MT. Nevertheless, the polyp loss percentage of both of the treatments was statistically significantly different from control, evidencing an effect. The fact that control individuals also exhibited polyp loss (19%) suggests that this species is challenging to keep under laboratory conditions and is prone to losing its polyps which might have enhanced the effect due to the sedimentations treatments.

Control individuals consumed significantly less food than MT individuals albeit having proportionally more polyps. Even if the difference in the $\Delta\ \delta^{13}C$ values used as a proxy for food intake are not statistically significantly different between the MT and GB treatments, it suggests that Red tree coral exposed to MT ate more than those exposed to GB. Higher food intake can be a way for compensating for higher metabolic expenditure. As seen in Fig. 20 the specimens produced mucus in order to clear out MT particles that settled in the tissue. This production has a metabolic cost for the corals (Riegl & Branch, 1995). The feeding strategy of Red tree coral, mucus net while having the polyp contracted (Fig. 9) makes it possible for the corals to still catch prey while protecting themselves from the sedimentation. Higher sedimentation rates and subsequent mucus production could exceed the ability of the corals to compensate the metabolic expenditure by feeding. The amount of sedimentation *P. resedaeformis* can tolerate while still being able to feed warrants more study. Higher sedimentation can also hampered the ability of the coral to reject the particles (Bak & Elgershuizen, 1976)

No mucus production was observed in GB treatment subjects suggesting that the particles did not attach to the tissue implying that they are less harmful than the MT particles. Also from the histological samples it was observed that just MT particles were embedded in the tissue (Fig. 25) which can leave the individuals prone to bacterial infection.

Primnoa resedaeformis is a broadcast spawner, excessive sedimentation can have repercussion for larval development, as shown for *L. pertusa* (Järnegren, Brooke, & Jensen, 2016; Larsson et al., 2013), Red abalone and Brown cup coral (Raimondi, Barnett, & Krause, 1997). Red tree coral larvae likely prefers rough surfaces for settling and are very sensitive in their early stages (Lacharité & Metaxas, 2013), hence benthic surface smothering, and turbidity can adversely affect recruiting.

5.3 General conclusions

After 3 months under sedimentation exposure no mortality was observed, suggesting that *D. florida* and *P. resedaeformis* can withstand moderate levels of turbidity (mean concentration of 18 mgL^{-1}) for a prolonged period of time (3 month). To the authors knowledge this time threshold has not been elucidated and longer periods might cause higher mortality. Since MT particles did penetrate tissue, further exposure to small angular particles (0-10µm) would be even more harmful.

As already determined for shallow water corals, sub lethal levels of stress can affect reproductive output (Michalek-Wagner & Willis, 2001; Ward, Harrison, & Hoegh-Guldberg, 2000), alter energy expenditure (Riegl & Branch, 1995) which is in accordance with the findings of this study.

In benthic habitats, soft cold-water corals are not just exposed to excessive sedimentation, other disturbances are also present (Ramirez-Llodra et al., 2011) which combined can have a synergistic effects for the overall fitness of the animal, minimizing their ability to cope by preventing homeostasis. For example, this study observed that *P.resedaeformis*, and *D.florida* are sensitive to prolonged elevated temperatures (above 9° C) (Appendix 2 A and B). Temperatures bordering the suggested 15° C upper lethal temperature limit for cold-water coral species (Brooke, Ross, Bane, Seim, & Young, 2013) that might arise due to global warming might affect the ability of these ecosystems to withstand other stressors. Average temperature are modelled to rice between 2 and 4.5° C (Stocker et al.,2013 (IPCC 2013)).

Global warming might also limit the availability of suitable food to cold-water coral ecosystems (Smith, De Leo, Bernardino, Sweetman, & Arbizu, 2008) because of maintained ocean stratification which decreases nutrient availability in surface water and then reduces fluxes of particle organic matter to the benthos leading to starvation. Hypoxia is also one of the possible consequences (Keeling, Körtzinger, & Gruber, 2010), and since no tolerance levels to hypoxia are known for cold-water corals besides *L. pertusa (Dodds, Roberts, Taylor, & Marubini, 2007)* it is difficult to predict what the consequences will be.

Ocean acidification might also have serious repercussions for soft-corals, even if they do not secrete a hard skeleton , they do use dissolved aragonite, and calcite for their supportive sclerites (Bayer & Macintyre, 2001).In their habitat suitability model for cold-water octocorals, Yesson et al. (2012) found aragonite and calcite saturation to be important parameters for single variable models reinforcing their importance for octocorals.

Anthropogenic sedimentation activity affects adult individuals, larvae and planulae which reduces the capacity of recovery. As mentioned above, suitable settling surfaces could be reduced because of particle smothering of bottom surface. Recovery rates of destroyed cold-water corals ecosystems after trawling efforts have been documented to be slow (Althaus et al., 2009; Kaiser et al., 2006; Roberts & Hirshfield, 2004).

5.4 Biological implications and extent of findings

The impacts of damaged cold-water coral habitats are numerous and can affect human kind in a large scale (food security can be compromised), limiting the possibilities for bioprospecting, reducing nutrient and element cycling).

These cold water benthic habitats are found worldwide and are associated with fish communities, specially *Sebastes* spp, Tusk (*Brosme brosme*) and Saithe (*Pollachius virens*) (Edinger, Wareham, & Haedrich, 2007; Husebø, Nøttestad, Fosså, Furevik, & Jørgensen, 2002; Jakobsen, 2016; Mortensen et al., 2015; Mortensen et al., 2010) (Fig. 26).These fish species are commercially important and even if the actual relation to coral habitat has not been completely elucidated (Auster, 2007) damaging

these habitats can have serious repercussion for fish populations, and fish landings. Fishermen in Norway noticed that fish landings diminished where coral habitats had been damaged (Fosså, Mortensen, & Furevik, 2002). Stronger evidence for the importance of cold-water corals for fish was discovered by Baillon, Hamel, Wareham, and Mercier (2012) classifying them as fish larvae nurseries.

Other associated fauna can be adversely affected by loss of coral surface ,Krieger and Wing (2002) documented fauna dependent on *Primnoa* spp and Mortensen and Mortensen (2005) reported several associated species.

Given the biodiversity associated to cold-water coral habitat,s it is to be expected that some species are more resilient than others, or that they react differently, such as *D. florida* and *P.resedaeformis* did to sedimentation and anthropogenic disturbances in this study. But the loss of certain species, and overall benthic biodiversity loss, affects deep-sea ecosystem functioning (Danovaro et al., 2008) which can affect us directly since the deep-sea ecosystems provides a number of services essential for our survival for a detailed description of the aforementioned services see (Armstrong et al., 2012; Thurber et al., 2014).

Mine tailings deposition has already been showed to reduce biodiversity in coral reefs in Papua Guinea (Haywood, Dennis, Thomson, & Pillans, 2016), in soft bottoms in northern Chile (Lancellotti & Stotz, 2004) and altered community structure in rocky subtidal bottoms in Northern Chile (González, Stotz, & Lancellotti, 2014). Effects on deep-sea habitats have also been documented (Hughes, Shimmield, Black, & Howe, 2015). Effects on cold-water coral habitats such as tissue smothering, behavioral changes, species composition have not yet been assessed.

The marked effect of increased sedimentation, specially of rough edged particles observed in the two model organisms in this study can be extrapolated to other soft corals found in cold-water environments. Mesophotic coral reefs have booth zooxanthellate and azooxanthellate corals (Lesser, Slattery, & Leichter, 2009), thus the information gathered here can also be used to asses risk for these type of ecosystems, which also are more exposed to terrestrial runoff sediment which is highly affected by climate change.

Future exploitation of deep sea resources such as deep-sea mining is imminent in the years to come (Mengerink et al., 2014; Ramirez-Llodra et al., 2011) and this endeavor will re-suspend sediments and create sharper particles. Possible exploitation of deep-sea methane hydrates (Krey et al., 2009) could also create massive sediment plumes in such events the results presented in this study can be used to asses risk to nearby coral communities.

5.5 Recommendations for submarine mine tailing disposal (STD), Deep-sea tailing disposal (DSTD) and Deep-sea mining industry.

Given the results obtained, recommendations regarding tailing disposal to the mining industry using STD, DSTD approaches, and for deep-sea mining endeavors are pointed with respect to the biology of the species studied and the observed effects from the sedimentation experiment.

When monitoring an affected area, the knowledge gathered about the coral biology could be helpful to determine the level of stress they are exposed to. *In situ* monitoring time lapse pictures could be used to assess polyp loss, which can be used as a proxy for stress levels in species similar to Red Tree coral.

Cauliflower coral changes stages (contracted, expanded (Fig.6)), as a part of their biology nevertheless if the amount of time spent totally contracted is long it could be interpreted as a sign of stress.

Processing particles to reduce their sharpness before disposal might reduce the inimical effects to the soft corals examined. Sharp particles appear more easily attached to the surface of the corals than smooth particles, and MT particles are therefore more difficult to reject with mucus transport, or by natural water flow.

The major particle size fractions in the coral tissue ranged from 0-10µm (histology samples) indicating that particles >10 um may be less harmful. Reducing this fraction size could also minimize harmful tailing effects on corals.

Sediment concentration outside the designated deposition zone should be minimized. Reducing the small particle fraction from the tailings would also help in this matter because the latter travels the furthest.
The results of this study showed that sedimentation stress did influence food intake in both species. Thus while monitoring, taking tissue sample from corals (or other type of biota) and analyzing them for stable isotope ratios $^{13}C/^{12}C$ and $^{15}N/^{14}N$ could reveal changes in diet, or food intake that may possibly be caused by sedimentation stress.

These recommendations can be further implemented in management policies for cold-water coral ecosystems. To the authors knowledge, the majority of management strategies so far, have been in regard to fishing efforts by closing the area (Roberts & Hirshfield, 2004),but stressors such as sediment plumes that can travel several kilometers have not been taken into consideration. The adaptive management strategy has been discussed by Jaeckel (2016) for policy making in deep-sea mining.

The results of this study that certain mine tailings sediments may have harmful effects. Due to the mineral composition of the ore extracted, toxicological effects to cold-water soft corals. Due to toxic elements or because of flocculation chemicals used may add additional stress to soft corals or other benthic organisms. Therefore, management decisions should also take into account the possible effects of toxicants as well as the physical effects as suggested by Moran, Reichelt-Brushett, and Young (2009).

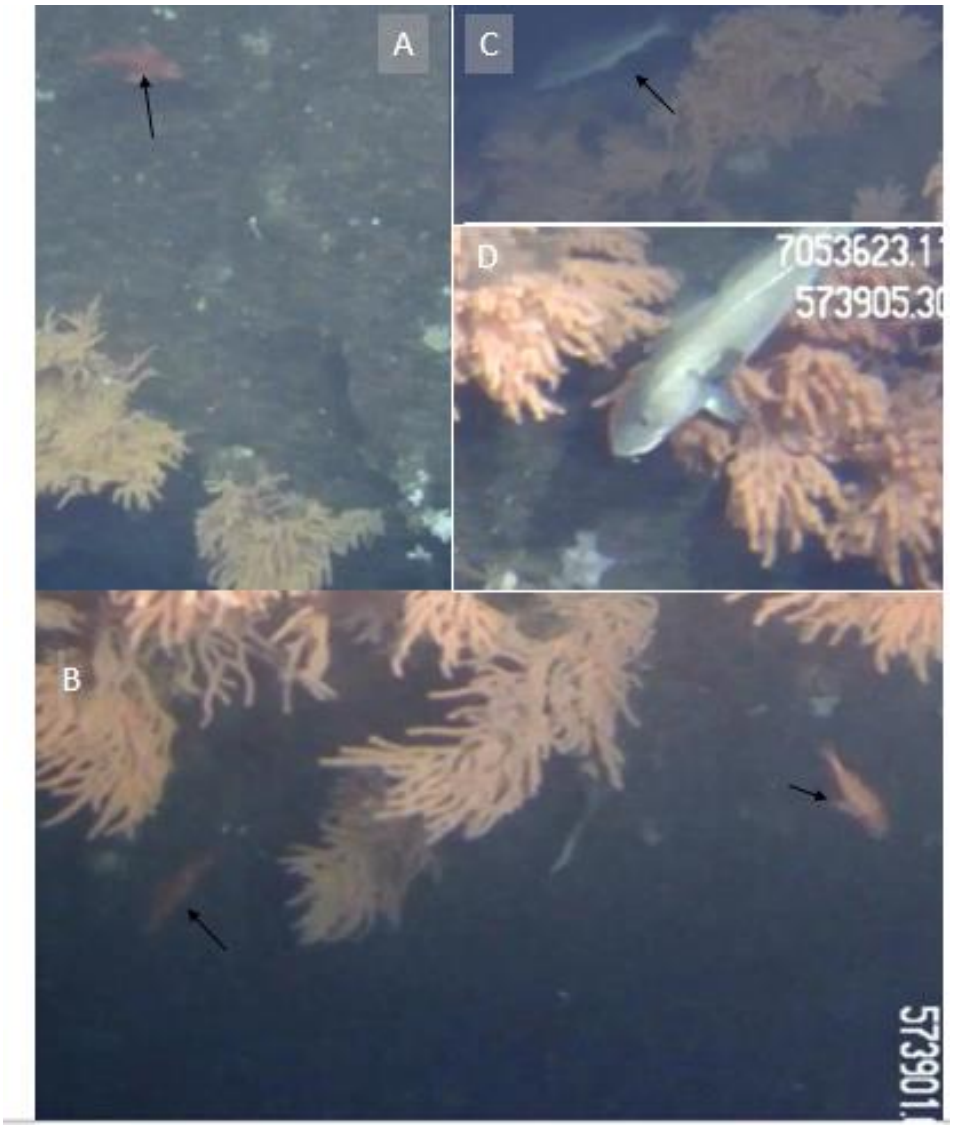

Figure 26: Fish observed with the ROV camera when collecting *P. resedaeformis* the 26th of June 2015. A: One Red rock fish (*Sebastes viviparous)* can be observed. B: two Red fish can be seen. C-D: Saithe and Atlantic cod in the coral habitat dominated by *P.resedaeformis*. (Fish pointed by arrows).

5.6 Limitations of the study and future prospects

Correctly evaluating the amount of sediments cold-water soft corals will be exposed to in their natural environments during deposition of mine tailings is very difficult since it depends on many factors: proximity to sediment pipe outlet, current speed and direction, type and amount of mineral tailings, site bathymetry and substrate type. Sediment concentration in the water column can be greater than those used in the present study. Also, mine tailing disposal is a continuous process lasting for several years, thus a 3-month period is not enough time to comprehend the total extend of the possible consequences. As mentioned before the behavior of soft corals *in situ* is not documented, thus their reactions might be different than the ones observed in this study.

More information about the general biology of cold-water soft corals is needed in order to properly understand their importance in the natural environment. For example, long term *in situ* monitoring, will be necessary to acquire data of a period where the corals are less vulnerable to stress so that dumping of tailings could be properly timed to limit the harmful effects, for example avoiding particle disposal when spawning episodes occur and periods of food scarcity. Similarly, better mapping of these ecosystems is recommended before delivering more STD licenses (which are given on a case basis in Norway (Kvassnes & Iversen, 2013) and allowing trawling. The Mareano-project in Norway (Mortensen et al., 2015) and the NOOA Deep-sea coral data portal in the USA have begun with this task (NOAA, 2016). Future research should also be aimed at understanding the reactions of cold-water corals to sedimentation to create appropriate thresholds that policy makers can use when making management decisions. As reviewed for tropical coral ecosystems by Erftemeijer, Riegl, Hoeksema, and Todd (2012), different coral assemblages in different natural environments react differently to sedimentation, as observed in the two species used for this study. Recovery rates of corals have not been established, which should be investigated in order to assess coral resilience, and to establish the possible length of sedimentation pulses and the time in between. Further on, bathymetry and geological studies should be recommended in each of the STD sites to properly model sediment, and sediment plume behavior in order to know

with some degree of certainty to how far they can reach, and how much sediments the ecosystems in question will be exposed to.

Lack of time and expertise prevented the author from analyzing in detail the histological samples which could have revealed valuable information about the cellular reaction of the corals to sedimentation as already done for other coral species (Peters, 1984; Vargas-Ángel, Peters, Kramarsky-Winter, Gilliam, & Dodge, 2007)

5.6.1 Experimental challenges

During the course of this study several challenges arouse:

It was difficult to obtain smooth edged sediments of the desired size. The GB was missing the smallest class size which might have had an effect on the corals reaction.

Programming the sediment delivery pumps was more difficult than expected and needed the help of an automation electrician. These two aspects delayed the start of the experiments.

The previously mentioned pumps often got clogged and did not delivered the expected amount of sediment slurry which prevented a concentration of 50 mg L^{-1} in each aquaria as was first intended. If the desired concentration would have been reached, more marked effects would likely have been observed.

The sediment mixing pumps were sensitive to the particles (mechanical wear and tear), especially to the mine tailings, and had to be replaced often, in total 11 pumps were used. If the damaged pumps were not discovered immediately, it meant a "break" in the sedimentation process.

Because of coral mortality before the experiment started not enough individuals were left in order to have a control group that did not receive ^{13}C enriched food.

Constantly monitoring the corals mimicking natural light conditions (dark) in the lab was impossible because of lack of a light source that did not disturbed the corals. Hence it was difficult to quantify the total amount of time that the Cauliflower corals remain contracted.

As different approaches were used to evaluate the effects of sedimentation stress in the model organisms, the time did not allow in deep analysis of each one of them.

5.7 Major conclusions

- Particles with rough edges may be more harmful to corals compared to round edged particles. Only small and rough edged particles (0-10μm) were found in different parts of the polyps indicating additional stress.
- Average particle concentration of 18 mg L^{-1} showed effects in both coral species, higher concentrations most likely will have more immediate, and serious effects on stress levels from which corals individuals might not be able to recover.
- Different corals species react differently to anthropogenic stressors. It is therefore important to make a proper impact assessment before embarking into deep-sea mining, mine tailing disposal in the sea floor or other exploitation endeavors. Continuous monitoring should also be established in order to have the necessary knowledge to manage and to obtain good decisions regarding habitat health.
- During the time-series experiments the two model organisms exhibited changes in their food intake likely due to the sedimentation stress measured by analyzing $^{13}C/^{12}C$ ratios. The different reaction observed in the two species could be explained by the different morphology of the polyp, and feeding strategy.

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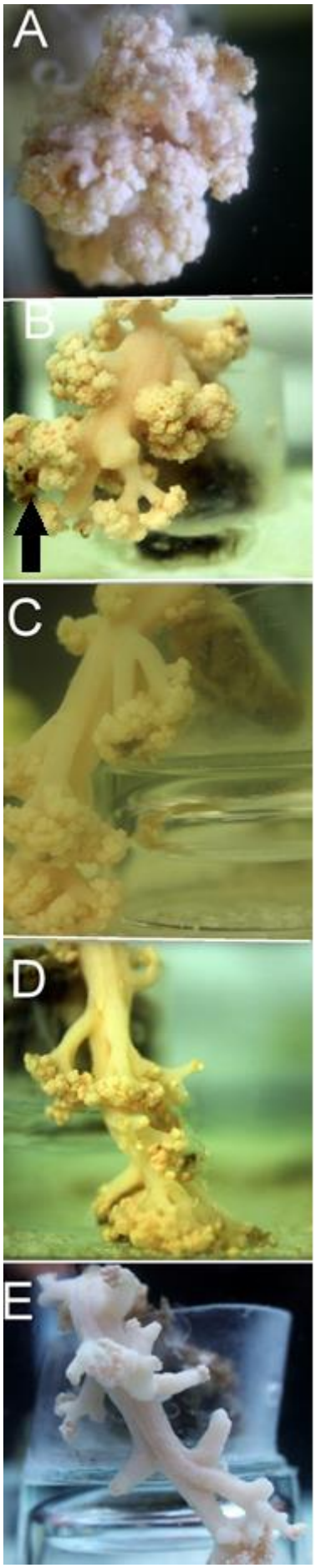
7 Appendices

7.1 Apendix 1: Detailed morphology of (A) *D. florida* and (B) *P. resedaeformis*

Appendix 1A: Detailed morphology of Cauliflower coral, note the pinnules in each tentacle pointed by the white arrows.

Appendix 1 B: Detailed morphology of Red tree coral polyp. Note the pinnules on the tentacles,one pinnule is pointed out by the with arrow. The sclerites are also visible as white scales in the polyp body, pointed out with black arrow.

7.2 Appendix 2: decaying individuals because of high temperature, and light flash (A) Cauliflower coral. (B) Red tree coral.



Appendix 2 A:

States of *Duva florida* when in poor physiological condition, presumably after exposure to temperatures > 9℃ for 2 weeks. The sick corals where generally characterized with tissue that was not as transparent as in healthy individuals. Most likely because the hydrostatic skeleton was not working properly.

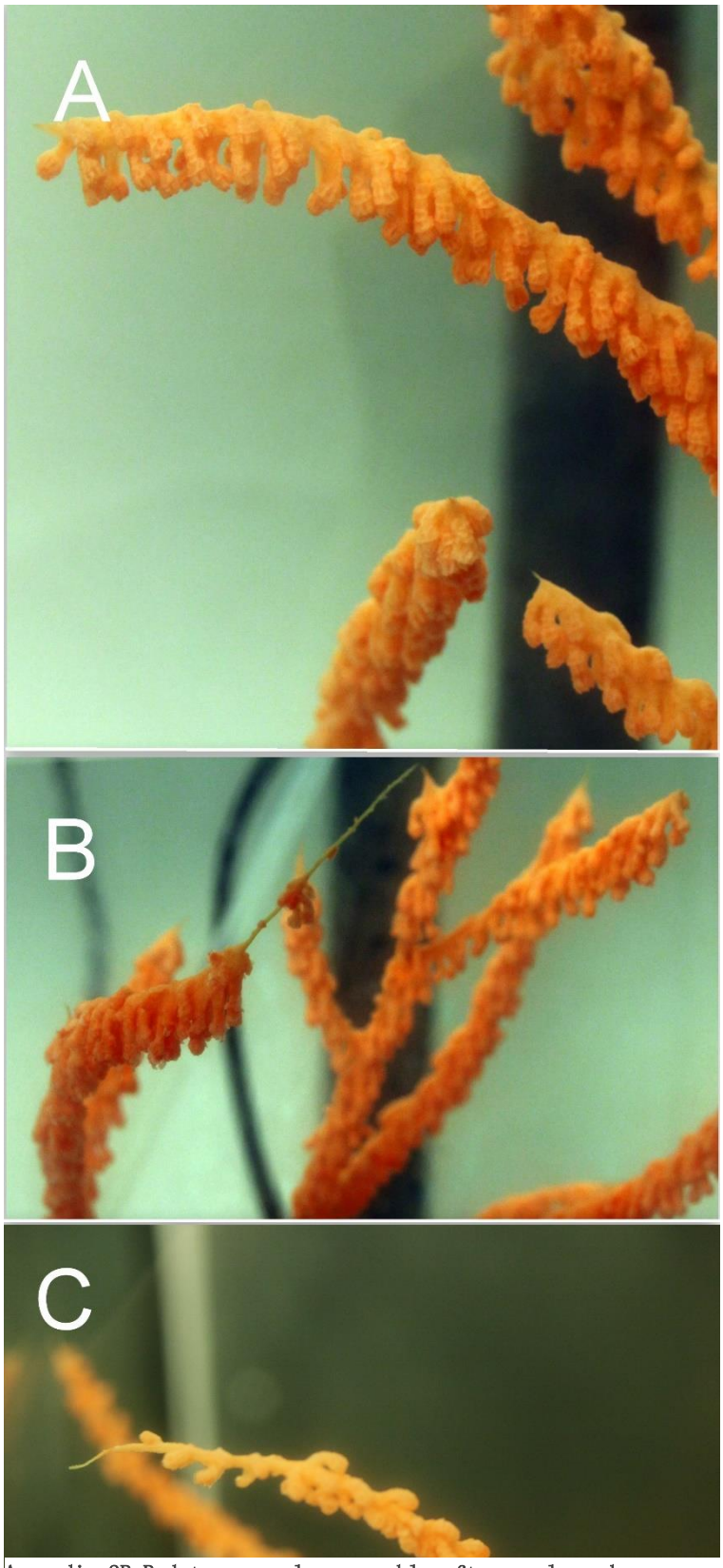
A: Contracted coral with polyps that are not as well defined as in healthy individuals.

B: Sick specimen expanding, the polyps do not expand totally with the rest body. Some necrosis can be observed, pointed by arrow.

C: The fully expanded coral. The polyps did not open, and some damage could be observed on the polyp tissue. At this stage, when disturbed the coral could still retract individuals.

D: Polyp loss could be observed. Some polyps became pinker.

E: Majority of the polyps had disappeared. Just the body remained lacking the ability to retract.



Appendix 2B:Red tree coral presumably after prolonged exposure to water temperatures above 9 ℃. A: At the beginning the polyps hanged from the skeleton. B: After 5 days hanging the polyps fell from the skeleton leaving it exposed. C: in this particular individual polyps got eroded away.