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DEPARTMENT OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

EFFECT OF SUSPENDED SEDIMENT ON EMBRYOS AND LARVAE OF THE COLD-WATER CORAL *LOPHELIA PERTUSA*

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Lophelia pertusa polyps, photo taken at Tjärnö Marine Laboratory

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Abstract

The cold-water coral *Lophelia pertusa* (syn. *Desmophyllum pertusum*) provides important habitats in deep-sea ecosystems, allowing for a biodiversity similar to that of tropical coral reefs. Anthropogenic activities like bottom-trawling and drilling however leads to increased concentrations of suspended sediment, which can potentially cause various negative effects in all life stages of corals. The purpose of this thesis was to study the effects of suspended benthic sediments on embryos and larvae of *L. pertusa*. The embryos/larvae were exposed to one of four concentrations of sediment (control, 2.5 mg/L, 5 mg/L & 25 mg/L), during one or three days. Assessments were made on embryonic and larval survival, embryo development and larval swimming speed. Exposure of the embryos started at around 1 day of age (6-30h after spawning), while larvae were either 5, 9 or 12 days old. "Embryos" where exposure started > 6h after spawning appeared to survive the highest sediment concentration for 1 day. However, a large proportion of them were still in the oocyte stage, indicating that either fertilization or development was stunned. After three days of exposure, the embryos had decreased survival rate and were more likely to lack cilia with increasing concentration of sediment. They also often responded by falling apart into multiple smaller embryo parts. Meanwhile, the larvae were mostly, but not entirely, unaffected in both their survival and swimming speed. These results suggest that the effect of suspended sediment depends on the age of embryos and larvae, the duration of sediment exposure, and the concentration of sediment. Future studies need to use more reliable techniques to be able to study large quantities of embryos, as well as other life-stages of *L. pertusa*, to further understand the effects of suspended sediment. Knowledge gained on this topic can aid management decisions, to mitigate potential adverse consequences on dispersal and deep-sea biodiversity.

Keywords: *Lophelia pertusa*, *Desmophyllum pertusum*, suspended sediment, embryo, larvae

Abstract (svenska)

Kallvattenskorallen; ögonkorall (*Lophelia pertusa*, synonym *Desmophyllum pertusum*) återfinns i djupa hav, där den bidrar med värdefulla habitat och en biodiversitet som kan jämföras med tropiska korallrev. Dock leder botten-trålning och oljeborrning till en förhöjd koncentration av suspenderat sediment, som i sin tur kan leda till diverse problem för korallen i alla dess livsstadier. Syftet med denna studie var att undersöka effekterna som av suspenderat bentiskt sediment har på embryon och larver av *L. pertusa*. Larver och embryon blev exponerade för en av fyra koncentrationer av sediment (kontroll, 2.5 mg/L, 5 mg/L & 25 mg/L), under en eller tre dagars tid. Mätningar utfördes på överlevnaden av embryon och larver, utvecklingen hos embryon och sim-hastigheten hos larver. Exponeringen av embryon började då de var runt en dag gamla (6-30h efter lek), medan larverna antingen var 5, 9 eller 12 dagar gamla. ”Embryon” vars exponering påbörjades > 6 h efter leken verkade ha överlevt den högsta koncentrationen av sediment under 1 dag. Däremot var en stor andel av dessa fortfarande i oocyt-stadiet, vilket tyder på en påverkan på antingen fertilisering eller utveckling. Efter tre dagars exponering hade embryon en minskad överlevnad och färre individer hade cilier i högre koncentrationer av sediment. De reagerade ofta även med att falla isär till ett flertal mindre embryodelar. Larverna var däremot mestadels, men inte helt, toleranta mot suspenderat sediment, både gällande överlevnad och sim-hastighet. Resultaten tyder på att effekterna av suspenderat sediment skiljer sig beroende på embryon och larvers ålder, längden av exponering, samt koncentrationen av sediment. Framtida studier behöver använda sig av mer tillförlitliga metoder för att studera stora mängder embryon, samt andra livsstadier hos *L. pertusa*, för att ytterligare förstå effekterna av suspenderat sediment. Denna nya kunskap kan guida förvaltningen av korallrev, samt förebygga negativ påverkan på ögonkorallernas spridningsförmåga och därmed gynna den associerade bio-diversitet som de härbärgerar.

Nyckelord: *Lophelia pertusa*, *Desmophyllum pertusum*, ögonkorall, suspenderat sediment, embryo, larv

1. Introduction

1.1. Background

Tropical, shallow-water coral reefs are known as biodiversity hotspots and have been targeted for research and conservation efforts for decades (Roberts et al. 2002). Less attention has been drawn to cold-water corals, which instead live in cold, deep waters and lack the symbiotic algae, zooxanthellae (Roberts et al. 2006). Like tropical corals, some cold-water corals are Scleractinian, which means they have a stony skeleton which can form reef frameworks that function as complex habitats for many other species (Roberts et al. 2006). Over 2700 species worldwide have been recorded in cold-water reefs, which makes their diversity comparable to the diversity in tropical coral reefs (Roberts and Cairns 2014). Only six of the known cold-water coral species are considered to have significant importance as widespread framework builders, and *Lophelia pertusa* (syn. *Desmophyllum pertusum*) is the most common of these (Roberts et al. 2009).

L. pertusa can cover large areas with a single clonal colony by using asexual propagation (Dahl et al. 2012). Being a gonochoric coral, each colony is either male or female (Waller and Tyler 2005). Sexual reproduction occurs by broadcast spawning over the span of two months, when oocytes and sperm are released into the open water, where fertilization then occurs (Larsson et al. 2014). The embryos are positively buoyant and reach the 64-cell stage after 48 hours. Cilia start to appear after 3-4 days, and the larvae start to swim at day 5. After 3-5 weeks they move downwards as they are ready to settle on a suitable surface (Larsson et al. 2014). Without settling, they can survive in the larval stage for 8-14 weeks, with some larvae surviving one year in a lab setting (Larsson et al. 2014; Strömberg and Larsson 2017).

L. pertusa has a world-wide distribution and is usually found at depths between 100-4000 m, in temperatures around 6°C (Davies et al. 2008). It is present in the Pacific Ocean, the Mediterranean Sea and the Indian Ocean, but most of the recorded data comes from the North-East Atlantic Ocean (Davies et al. 2008). Many of these recordings come from the Norwegian continental shelf, which is home to *L. pertusa* reefs that stretch over several kilometers (Fossa et al. 2002).

However, anthropogenic activities such as bottom-trawling and hydrocarbon drilling pose a severe threat to these reefs (Roberts et al. 2006). On the Norwegian continental shelf, 30-50% of the reefs are estimated to be damaged, likely due to physical impact from trawling (Fossa et al. 2002). The Skagerrak area outside Norway has the highest intensity of trawling in Europe, where large areas are commonly swept to a depth of 500 m (Eigaard et al. 2017). Additionally, these activities lead to increased levels of suspended sediments in the water, which is also of concern for the health of corals (Jones et al. 2015). While MPAs and no-trawl zones might protect areas from direct damage, trawling outside of a protected area could still lead to increased levels of suspended sediment inside the area (Grant et al. 2019). Fine particles can follow the current for several

kilometers before deposition and double the background concentration 2 km away (Grant et al. 2019; Purser 2015).

1.2. Suspended sediment as a threat to corals

Most of the research on the effect of suspended sediment has been performed using warm-water coral species. Negative effects have been found on several stages of their life-cycles (Tuttle and Donahue 2022). In adult corals, effects include a decreased growth rate, tissue mortality and other sublethal effects related to the aspects regarding photosynthesis (Tuttle and Donahue 2022), the latter which is not relevant for cold-water corals.

In general, corals in their early life-stages are more sensitive to sediment than adults (Fabricius 2005). Several studies report on a reduction in fertilization rate and decreased larval settlement as a response to suspended sediment (Erftemeijer et al. 2012; Gilmour 1999; Hodgson 1990; Humphrey et al. 2008). Meanwhile, no effect has been found on survival or development rate in embryos (Erftemeijer et al. 2012; Gilmour 1999; Ricardo et al. 2016). Embryos have been seen encased in mucus and sediment, which may have protected them from damage, but also caused them to sink during their otherwise buoyant phase (Ricardo et al. 2016). The encased embryos kept developing and later emerged as larvae by using their cilia movements to remove sediment. Research on larval survival has seen contrasting responses. While one experiment showed that larvae exhibited tolerance to extreme sediment concentrations, another lost a large majority at 18 times lower concentrations (Gilmour 1999; Ricardo et al. 2016). These kinds of differences may be explained by variations in study designs. It is likely that the effect of sediment depends on coral species, duration of exposure, amount, as well as the type of sediment and its content (Fabricius 2005).

1.3. Effects of suspended particles on *Lophelia pertusa*

Adults of *L. pertusa* seem tolerant to suspended particles consisting of either natural sediments or drill cuttings. After several weeks of exposure to either sediment type, there was still no dramatic differences in polyp mortality or skeletal growth (Baussant et al. 2018; Larsson et al. 2013).

Research on early stages of *L. pertusa* is limited to three studies with varying results. In a pilot study, Larsson et al. (2013) saw a significant decrease in larval survival rate, when subjected to suspended drill cuttings for 5 days. On the other hand, Järnegren et al. (2017) found the larvae to have high survival rates, even in several times higher concentrations of drill cuttings. Although, the duration of exposure was only 24 hours. They also found an age-dependent effect, with younger larvae responding with a decreased survival in concentrations that older larvae were able to tolerate. By comparing drill cuttings with other materials that are dispersed during drilling events, Järnegren et al. (2020) confirmed

that the response to suspended particles depends on the material. Apart from certain decrease in survival, larvae in all treatments were seen with varying amount of particles attached to them, while showing reduced or complete incapability to swim. After being allowed to recover in clean water, most larvae would lose the particles and regain their swimming abilities.

While these results are species-specific, they may not correspond to the effects of natural benthic sediment on either larvae or embryos. It is important to fill these knowledge gaps to improve management of *L. pertusa* and the multitude of species that are depending on it.

1.4. Aim of study

The objective of this study is to examine the effects of suspended benthic sediments on embryo and larvae of *L. pertusa*. More specifically, to test (1) if the survivorship will decrease for both embryos and larvae, (2) if the embryo development will be disrupted and (3) if the larval swimming speed will decrease. Three concentrations of suspended sediment (2.5 mg/L, 5 mg/L & 25 mg/L) will be used on embryos and larvae of different ages to highlight any concentration-dependent and age-dependent effects. The effect of the duration of exposure will also be evaluated by comparing short-term (~1 day) with long-term (~3 days) exposure.

2. Method

2.1. Experimental preparations

2.1.1. Corals

Adult corals were sampled at two occasions from the Tisler reef, in Skagerrak, in December 2021 and in January 2022. Fragments of adult corals were collected using a remotely operated vehicle (ROV) at depths around 100 m. They were then kept at the Tjärnö Marine Laboratory, a research station belonging to the University of Gothenburg, where all experiments took place. Male and female corals were kept together in aquariums with flow-through seawater (filtered to 5 µm) at a temperature of 8°C. Multiple spawning events occurred between January 26th to March 17th, 2022. After each spawning event, water containing the gametes were siphoned from the aquariums and stored in glass bottles for later use.

2.1.2. Sediment

Natural benthic sediment was collected on January 25th, 2022, near the Koster islands, Sweden (58.909283 N, 11.057153 E), around 10 km south of the Tisler reef. The sediment was collected with a van veer grabber at 138 meters depth. The sediment was then wet sieved with seawater to the size of ≤ 63 µm. The sediment particles were allowed to settle, after which the clear water above was removed by siphoning. The sieved sediment was kept in a bucket with a lid in a cold room at 0°C throughout the experiment period.

The dry weight-wet weight ratio was measured in order to extrapolate the amount of wet sediment needed for the experiment concentrations. Four sediment samples were weighed and washed with deionized water while suction filtering (Whatman glass fiber filter, GF 6, 47 mm). The samples were then dried at 60°C overnight and weighed again. The mean dry weight-wet weight ratio was 0.18 (+/- < 0.002).

Three bottles with different sediment concentrations were prepared before each experiment. These had a higher concentration, so that 10 ml of each mixture would result in the goal concentrations (2.5 mg/L, 5 mg/L and 25 mg/L respectively), when later diluted in the sample flasks. These concentrations were chosen to ensure compatibility with a previous pilot study on *L. pertusa* larvae (Larsson et al. 2013), and to resemble in situ concentrations near trawling and drilling events (Arjona-Camas et al. 2021; Purser 2015; Ricardo et al. 2016).

2.1.3. Sample flasks

A small test was performed without embryos or larvae to compare the suitability of two types of available sample flasks: VWR surface treated tissue culture flask, 50 ml (75 ml when full) and Cellstar suspension culture flask, 50 ml (62 ml when full). Four of each flask type were filled with the highest sediment concentration and placed on a rotating plankton wheel for ~24 hours (Fig. 1A). The Cellstar flasks were easy to close without trapping any air bubbles but accumulated many lumps of clustered sediment during the test run. The VWR flasks were therefore preferred, since only a few lumps were seen. On the other hand, these flasks were unfortunately impossible to close without trapping an air bubble (Fig. 1B).

Unused sample flasks were soaked in freshwater to remove any chemical residue. For later experiments, the sample bottles were reused after being rinsed repeatedly and soaked in hot freshwater.

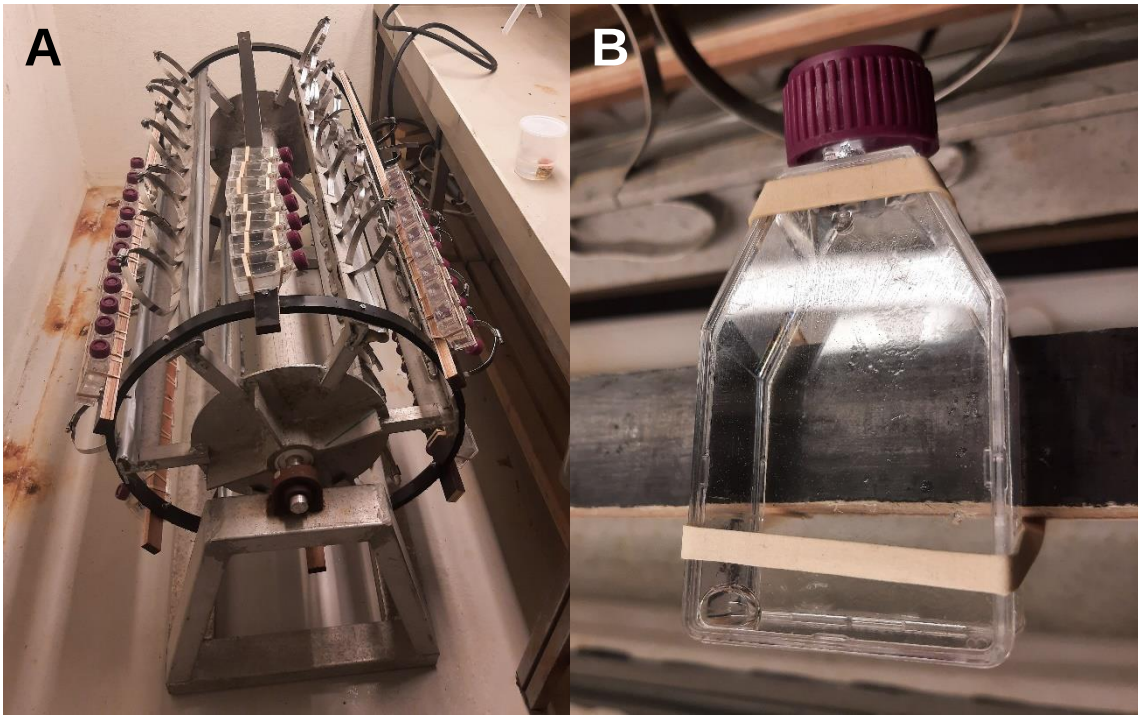

Figure 1. Setup for sediment exposure of *Lophelia pertusa* embryos and larvae. A) Plankton wheel with several sample flasks during sediment exposure. **B)** Sample flask (VWR surface treated tissue culture flask) containing 75 ml water attached to the plankton wheel with a rubber band. A large air bubble is seen in the bottom left corner. This sample contains freshwater only for the sake of demonstration, and no embryos/larvae or sediment.

Table 1. Overview of all experiments on *Lophelia pertusa* embryos and larvae. The age of embryos/larvae is indicated in days. Exact age of embryos in hours within parenthesis. Each experiment was conducted on separate occasions with different spawn batches, apart from those marked with an asterisk (*). All spawning occurred during 2022.

Experiment	Age during treatment	Spawn date	Survival	Develop-ment	Swimming speed
Short-term exposure	1(>6h)-2	15/3	x	x	
Embryo trial 1	1(6h)-4	23/2	x	x	
Embryo trial 2	1(30h)-4	26/2	x	x	
Embryo trial 3	1(22h)-4	17/3	x	x	
5-day old larvae trial 1	5-8	3/2 *	x		x
5-day old larvae trial 2	5-8	28/2	x		
9-day old larvae	9-12	29/1	x		
12-day old larvae	12-15	3/2 *			x

2.2. Experimental design and setup

The experiments were conducted between February 7th and March 18th, 2022. The experimental trials were started on separate occasions and usually with different spawn batches (Table 1). Embryos were exposed to the sediment treatments starting at roughly one day's age (6-30 hours after spawning) and received either one or three days of exposure. Larvae were of three different ages and all trials received three days of exposure. The larval experiments will be referred to by their age at the start of the exposure (5, 9 & 12 days old). In total, 8 experimental trials were performed, where embryos and larvae of different ages were exposed to one of four sediment treatments – control, 2.5 mg/L, 5 mg/L or 25 mg/L. Due to a smaller spawn batch, only control and 25 mg/L were used in the short-term exposure experiment.

The experiments were generally prepared and exposed to the treatment in the same way, but with different types of assessments made afterwards. Assessments were either on survival, development, or swimming speed. When two types of assessments were made on the same day, the same spawn batch and some of the same samples were used for both assessments.

2.2.1. Setup for sediment exposure

Embryos or larvae were added to sample flasks containing ~30 ml (+/- 10ml) seawater (filtered at either 1 or 5 µm). The sample size varied between 13-52 individuals per flask and each treatment had 3-5 replicates, depending on the available amount from the spawn batch (Appendix B1). For the first experiment, larvae were picked out from the stock under a stereo microscope, to avoid picking abnormal individuals or debris. Due to time constrains, picking was later done with the bare eye in a dark room with a flashlight to light up the embryos. Sediment mixture was taken from one of three prepared concentrations. 10 ml of sediment mixture was added to each sample flask, except those in the control group. All sample bottles, including control were then topped off to the brim with filtered sea-water. To try to avoid air bubbles that could affect embryos and larvae, the sample bottles were slightly overfilled and lost 1-2 ml water when the cap was put on. Yet, the air bubble could not be avoided completely. The sample bottles were then secured to a plankton wheel (diameter ~56 cm), which rotated at 45 seconds per turn to keep the sediment suspended. The samples were kept at a temperature of 8°C, which corresponds to the temperature *in situ* during the spawning season (Larsson et al. 2013), for the duration of the exposure, either 1 or 3 days.

2.2.2. Embryonic and larval survival

To measure survival rate, the proportion of survivors in each sample flask were counted under a stereo microscope. All remaining individuals were considered to be alive, since they usually dissolve quickly when dead (Larsson et al. 2013).

The embryos were observed to be in a wide range of sizes and could sometimes be more abundant than at the start of the experiment. This was likely due to embryo cleavage,

a phenomenon known to occur in embryos in early development, which leads to multiple smaller embryos (Heyward and Negri 2012). Since each cleavage could have resulted in two or more small embryos, it was not possible to determine how many of the original embryos they derived from. The embryos were therefore divided into two size classes, large and small, by comparing their relative size to each other. Survival rate was based on the proportions of large survivors out of the total number of embryos before sediment exposure. The smaller embryos were counted up to 50, due to the difficulties of counting all. As an indication of the extent of embryo cleavage, the proportion of small embryos were then obtained from the total survivors (large and small). Due to the uncertainty of exact numbers above 50, all proportions of small embryos above 90% were adjusted to 90%.

For larval survival, all individuals of any size were counted as a survivor. To check the validity of the size classification, the diameter of some of the embryos was measured in a phase-contrast microscope at 40x zoom.

2.2.3. Embryo development

A random subsample of the survivors was taken from the flasks and observed under either a phase-contrast or compound microscope. The number of individuals per sample and number of replicates that were analyzed depended on the amount of embryos that survived the experiment and being handled (Appendix B2). Smaller embryos often dissolved or disappeared during handling, so development was mainly checked on larger embryos.

For the short-term exposure experiment, the embryos were categorized as either oocytes (Fig. 2A), in the 2-cell stage (Fig. 2B) or in a higher cell-stage with more than two cells (e.g., Fig. 2C). Perfectly round embryos without cilia were assumed to be oocytes due to their age. Notes were also made on whether or not there was sediment stuck to the embryos.

In the 3-day exposure experiments, the embryos were categorized as either having less than 64 cells (Fig. 2B-C) or being in/above the 64-cell stage (Fig. 2D-E). Perfectly round embryos without cilia were assumed to have above 64 cells, due to their age and knowing that undeveloped oocytes rarely survive for 3 days at 8°C (A. Larsson, personal communication, 2022). The occurrence of any cilia was also noted.

2.2.4. Larval swimming speed

Larvae were photographed using Nikon D5600 with a macro lens, mounted on a tripod. The photographs were taken in the thermo-constant room with the room lighting off. Portable lamps and a black background were set up to easily distinguish the larvae.

For the first trial, each sample flask was taken off the plankton wheel and left standing up for two minutes to let the sediment settle. 5 photographs were taken with 10 seconds interval. A ruler appeared in each photograph, to indicate the scale at the central part of the flask.

To avoid taking pictures where settling sediment could affect larval movement, another approach was used for the second trial. A plastic tank (11x6x22 cm) was filled with

(900 ml +/- ca 50 ml) of seawater (filtered at 5 µm). The water was prepared the day before and kept in the same room to reach the same temperature of 8°C. For each sample, a ruler was photographed at the front and back of the tank, so the mean of the scale could be used for later calibration. Multiple larvae (~10) from the same sample flask were added to the tank all at once, using a long pipette. Since larvae of this age swim upwards (Larsson et al. 2014; Strömberg and Larsson 2017), they were carefully dropped a few centimeters above the bottom by slowly moving the pipette sideways in a straight line, while avoiding getting too close to the walls. Immediately after, 13 photographs were taken with 10 seconds interval. The tank was rinsed and filled with new seawater before each sample.

The image sequences were analyzed using the plugin *Manual tracking* in the software ImageJ (version 1.53k). The scale was calibrated for each sample based on the pictures with the ruler. Anything that was moving straight down was excluded and presumed to be sediment that was settling. The position of each larva was tracked at each time point and resulted in an output with data on their speed between each time point. The mean swimming speed (µm/s) was calculated for each sample flask. The number of tracked individuals per sample varied, since not every larva would swim (Appendix B3).

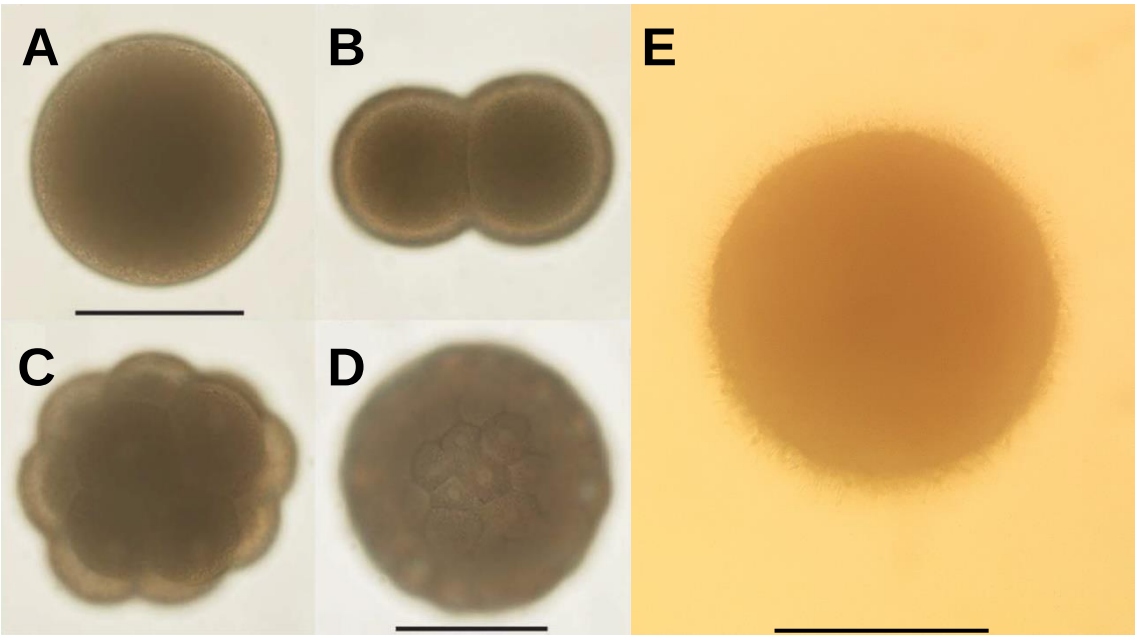

Figure 2. Normal development stages of *Lophelia pertusa* embryos. A) Oocyte. **B)** 2-cell stage (6h). **C)** 16-cell stage (24h). **D)** 64-cell stage (48h). **E)** Larvae with cilia (4 days). This larva was from the 2.5 mg/L treatment group in the embryo experiment, trial 1. It is representative of the expected development at that age. Pictures A-D are modified from Larsson et al. (2014). Picture E was taken through a microscope. Scale bar 100 µm.

2.3. Statistical analyses

The data was analyzed using the program R, with RStudio (version 4.1.1) as interface. All plots were made using the package *ggplot2*. A type I (α) error rate of 0.05 was used for all statistical analyses. For every dataset, the normality of residuals was tested with a Shapiro-Wilks test and homogeneity of variances was both visually analyzed and tested with Levene's test. If the assumptions for parametric tests were fulfilled, the datasets were analyzed with either a two-sample t-test or a one-factor ANOVA with sediment treatments (n=4) as factor. The ANOVA was followed by a Tukey's post-hoc test. Several of the datasets suffered from a floor/ceiling effect which caused deviations in variance and was instead tested with non-parametric Kruskal-Wallis tests. This was followed by Dunn's tests for multiple comparison using the Benjamini-Hochberg adjustment, from the *FSA* package in R. If significance was found in either case, the effect size was extracted using the Cohen's D method from the R-package *effsize*.

2.3.1. Embryonic and larval survival data

The survival rate for the short-term exposure embryo experiment only had two treatment groups: control and 25 mg/L of suspended sediment. The data was analyzed with a two-sample t-test to compare the mean survival rate between the groups.

The initial plan was to analyze the 3-day exposure embryo experiments using a two-way ANOVA, with trials (n=3) and sediment treatments (n=4) as factors. The interaction was of interest since it could indicate changes of the effect of sediment with time or differences in sediment sensitivity among spawn batches. However, the assumptions of an ANOVA were not fulfilled, even after the data was transformed using log, square root, cube, or reciprocal transformations. Instead, each experiment trial was tested separately using a Kruskal-Wallis test. The difference between each trial was also tested by comparing only the control groups' survival rates, using a one-factor ANOVA. The intention of which was to find out if the baseline survival rate differed between spawn batches. The interaction between trial and treatment was analyzed graphically. The proportion of small embryos had a ceiling at 90% and was therefore not used for inferential statistical analyses, but also visualized in a graph.

The two trials of experiments on 5-day old larvae were tested separately, due to the large differences seen when plotted. The first trial was tested with a one-factor ANOVA. The second trial, as well as the data on 9-day old larvae were tested with Kruskal-Wallis tests. Again, the effect of spawn batches was tested by comparing the controls from the two trials on 5-day old larvae. This was done with a two-sample t-test.

2.3.2. Embryo development data

A two-sample t-test was performed to compare the proportion of oocytes between the control and the 25 mg/L treatment in the short-term experiment. The proportions of embryos in the 2-cell stage and those with more cells were not analyzed since the proportions of development stages are not independent from one another. There was also a lack of

replicates in the development part of the 3-day exposure embryo experiment. These datasets were presented graphically.

2.3.3. Larval swimming speed data

For both experiments on larval swimming speed, a one-factor ANOVA was used to compare the mean swimming speeds (μm/s) among the four treatments.

3. Results

3.1. General observations

After the 24 hour test run of sample flasks without embryos or larvae, there were minimal sediment lumps (~1 mm) in the highest concentration. However, during experiments with embryos or larvae, sediments lumps were abundant and occurred in almost every sample flask with the highest concentration. Sometimes, a few lumps were seen in lower concentrations. They seemed to accumulate during the exposure as there were more after 3 days than after 1 day, but remained suspended while on the plankton wheel. When sediment lumps were picked up with a pipette and placed in another jar with filtered seawater, they sometimes loosened and revealed an embryo within. This was only seen in the short-term experiment on embryos. In higher concentrations of sediment, embryos were often found with more or less sediment attached to them (Fig. 3A).

While determining the development stages, sometimes embryos in various cell stages (2-64) had loosely jointed cells (Fig. 3B). After returning to the sample after a few minutes or hours, many smaller embryo parts were found instead (Fig. 3C).

There was a large size difference between embryos in the embryo experiments and almost every sample contained some embryos that were classified as small. As previously mentioned, some of the samples contained more embryos at the end of the experiment than at the start. Smaller embryos often looked less dense and almost see-through. During handling, they would easily dissolve, making it difficult to observe them under a microscope. Embryos classified as large ranged between 100-183 μm (usually around 150 μm); those classified as small ranged between 23-108 μm. The slight overlap between these scales highlights the difficulties in categorizing certain embryos by measuring by eye. Although, most embryos were either distinctly small or distinctly large.

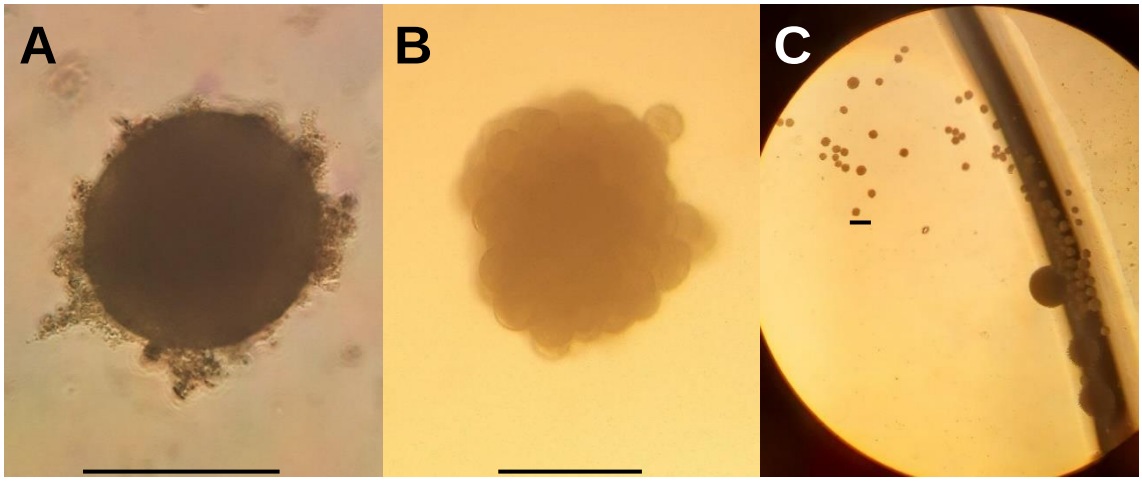

Figure 3. Abnormal development of *Lophelia pertusa* embryos/larvae. A) Embryo/larvae encased in sediment (4 days). Found in a sample treated with 25 mg/L of suspended sediment, in the embryo experiment trial 1. **B)** Embryo in the 64-cell stage (4 days), consisting of loosely attached cells that a few minutes later turned into **C)** separated cells. Found in a sample treated with 2.5 mg/L of suspended sediment, in the embryo experiment trial 1. Scale bar 100 µm.

3.2. Embryonic and larval survival

The embryo experiments showed different responses depending on duration of exposure (Fig. 4). The short-term exposure did not lead to any difference in survival rate between embryos treated with 25 mg/L of sediment and the control group (two-sample t-test, $t(4) = -0.93$, $p = 0.41$). Meanwhile, all trials in the 3-day exposure embryo experiments showed a general pattern of decreasing survival rate with increasing sediment concentration. Although, this difference in survival rate was only significant in one of the trials (Kruskal-Wallis tests, trial 1: $H(3) = 6.45$, $p = 0.09$; trial 2: $H(3) = 10.14$, $p = 0.02$, Table 2A; trial 3: $H(3) = 6.72$, $p = 0.08$). In all trials, there was an occurrence of samples with 0% survival rate, mainly found in the 25 mg/L sediment treatment group. In the third trial, one extreme outlier in the highest concentration raised the mean.

The proportion of small embryos out of the total survivors showed a pattern opposite to that of the survival rate of large embryos (Fig. 5). In the short-term experiment, the proportion of small embryos was higher in the control group than in the 25 mg/L treatment, although the variation was large between the samples. Among the 3 trials of 3-day exposure embryo experiments, the control group was typically dominated by large embryos, while the higher concentrations had a large proportion of small embryos.

Among the 3-day exposure experiments on both embryos and larvae, the effect of sediment was in general stronger at younger ages (Fig. 4). Apart from the clear trends in the embryo experiments, the second trial on 5-day old larvae also found a decreased survival rate with increasing concentration of sediment (Kruskal-Wallis test, $H(3) = 9.47$, $p = 0.02$, Table 2B). In this trial, the overall survival was low and resulted in no survival in the two highest concentrations. The first trial on 5-day old larvae and the experiment on

9-day old larvae showed no significant difference in survival (one-factor ANOVAs, 5-day old: $F(3,16) = 2.79$, $p = 0.07$; 9-day old: $F(3,8) = 1.32$, $p = 0.33$).

There were differences in the baseline survival rate between spawn batches, when comparing trials from the same age group. The control groups in the 3 trials of the embryo experiment differed significantly in survival rate (one-factor ANOVA, $F(2,8) = 12.75$, $p = 0.003$). There was no difference between the first 2 trials (Tukey's test, $p = 0.998$), while the third trial had a significantly higher survival rate than both the previous two (Tukey's test, trial 3-1: $p = 0.005$; trial 3-2: $p = 0.005$). The effect sizes were high between both comparisons (Cohen's d, trial 3-1: d = 3.02 CI [0.17-5.88]; trial 3-2: d = 5.60 CI [1.28-9.92]). When comparing the control groups from the experiments on 5-day old larvae, the first trial had significantly higher survival rate than the second trial (two-sample t-test, $t(6) = 6.68$, $p < 0.001$), with a large effect size (Cohen's d = 4.88 CI [1.40-8.36]).

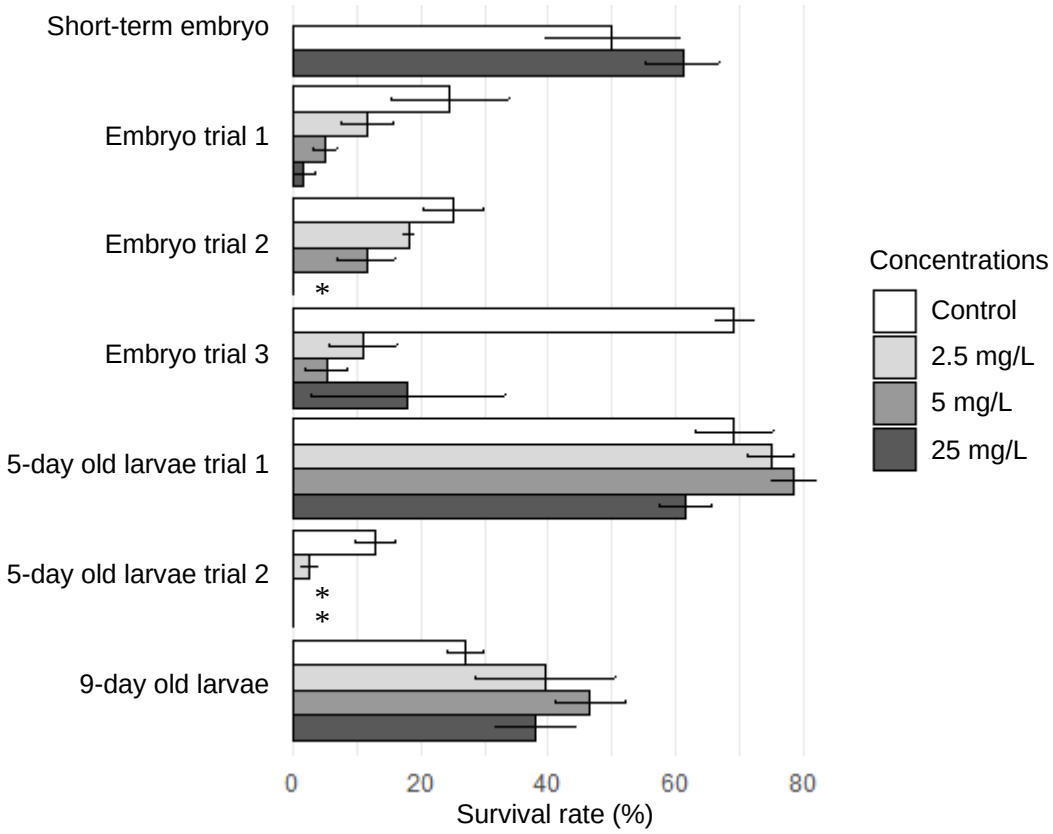

Figure 4. Survival rate (mean +/- SE) in *Lophelia pertusa* embryos and larvae of various ages after being exposed to different concentrations of suspended benthic sediment. Survival rate is the proportion of large embryos or large + small larvae after exposure, compared to before. The larvae experiments are named after the larvae age at the start of exposure. Embryos started exposure at around 1 day of age with some variations: > 6h (short-term), 6h (trial 1), 30h (trial 2) and 22h (trial 3). See table 1 for more information on the experiments. Asterisk (*) for significance ($p < 0.05$) compared to control. Replicates in Appendix B1.

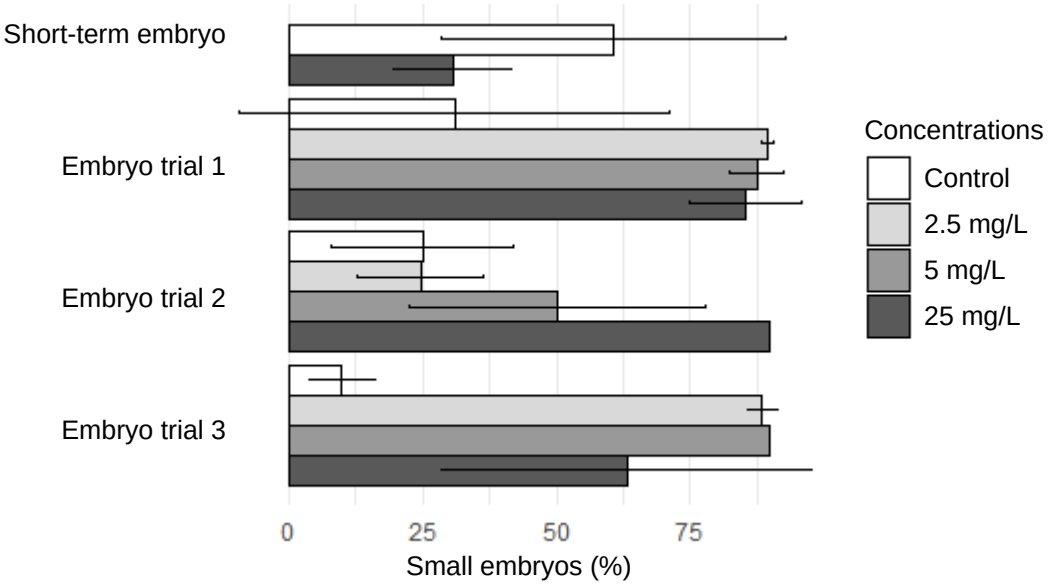

Figure 5. Proportion of small *Lophelia pertusa* embryos (mean +/- SD) among the total survivors after exposure to different concentrations of suspended benthic sediment. See table 1 for more information on the experiments. All values above 90% were adjusted to 90%, since the number of small embryos were only counted up to 50 per sample. Replicates in Appendix B1.

Table 2. Post-hoc results from sediment experiments on *Lophelia pertusa* embryos and larvae. Comparisons are made between each pair of the four sediment treatments (control, 2.5 mg/L, 5 mg/L, 25 mg/L). Adjusted p-values from Dunn's test for survival rate in **A)** embryo experiment trial 2 and **B)** 5-day old larvae trial 2. **C)** p-values from Tukey's test for swimming speed in 5-day old larvae trial 1. Effect sizes in parentheses for the significant comparisons (Cohen's d, CI).

Comparisons (mg/L)	**A**	**B**	**C**
Control - 2.5	0.45	0.26	0.46
Control - 5	0.19	0.048 (3.35, -0.16-6.87)	0.88
Control - 25	0.02 (3.80, 0.90-6.69)	0.02 (3.35, -0.16-6.87)	0.30
2.5 - 5	0.44	0.38	0.88
2.5 - 25	0.07	0.31	0.03 (2.27, 0.005-4.53)
25 - 5	0.26	1.00	0.11

3.3. Embryo development

After the short-term exposure to suspended sediment, there were notable differences in embryo development (Fig. 6). Embryos exposed to 25 mg/L of sediment were dominated by oocytes (M = 90.28, SE = 5.01), while the control group had a significantly lower proportion of oocytes (M = 29.44, SE = 2.42), (two-sample t-test, $t(4) = -10.94$, $p < 0.001$). There was no dramatic difference in proportion of embryos in the two-cell stage between the 25 mg/L treatment (M = 9.72, SD = 8.67) and the control group (M = 22.22, SD = 25.46). Embryos with more than 2 cells were only observed in the control samples, but varied in abundance among replicates (M = 48.33, SD = 27.54). The exact cell stage in this category ranged between 3-17, median number of cells out of those with more cells were 13 cells. In the 25 mg/L treatment, 83% of the analyzed embryos had sediment attached to them.

Data from the 3-day exposure embryo experiments lacked replicates and was not analyzed using inferential statistics. Results show that the occurrence of embryos that had reached the 64-cell stage was similar in all concentrations (Fig. 7). There is however a trend of fewer embryos with cilia in higher sediment concentrations (Fig. 8). All embryos with cilia had also reached the 64-cell stage. Embryos were also seen with attached sediment in these experiments, but the prevalence was not counted (Fig. 3C).

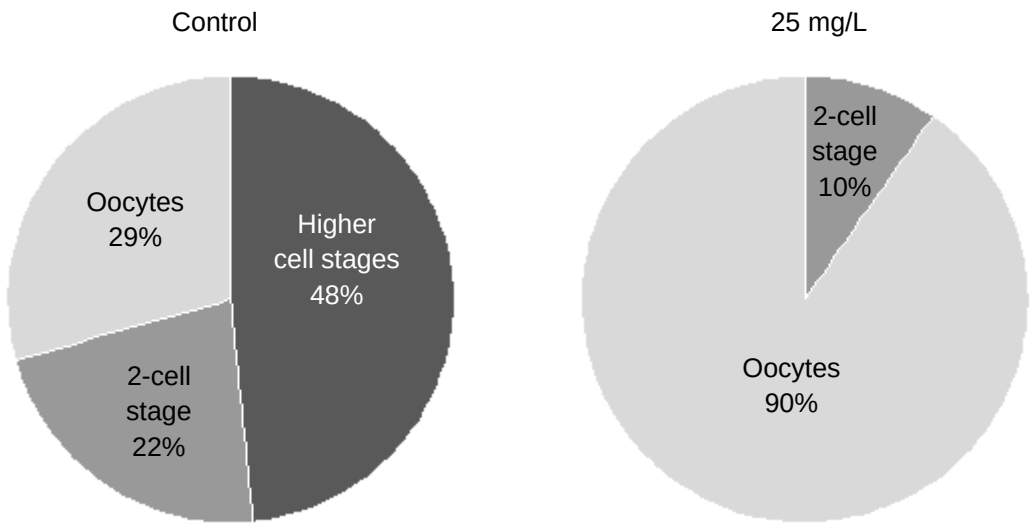

Figure 6. Development stages in the short-term *Lophelia pertusa* embryo experiment. Mean proportions of developmental stages after 1 day exposure to 25 mg/L of suspended benthic sediment compared to control. See table 1 for more information on the experiment. Replicates in Appendix B2.

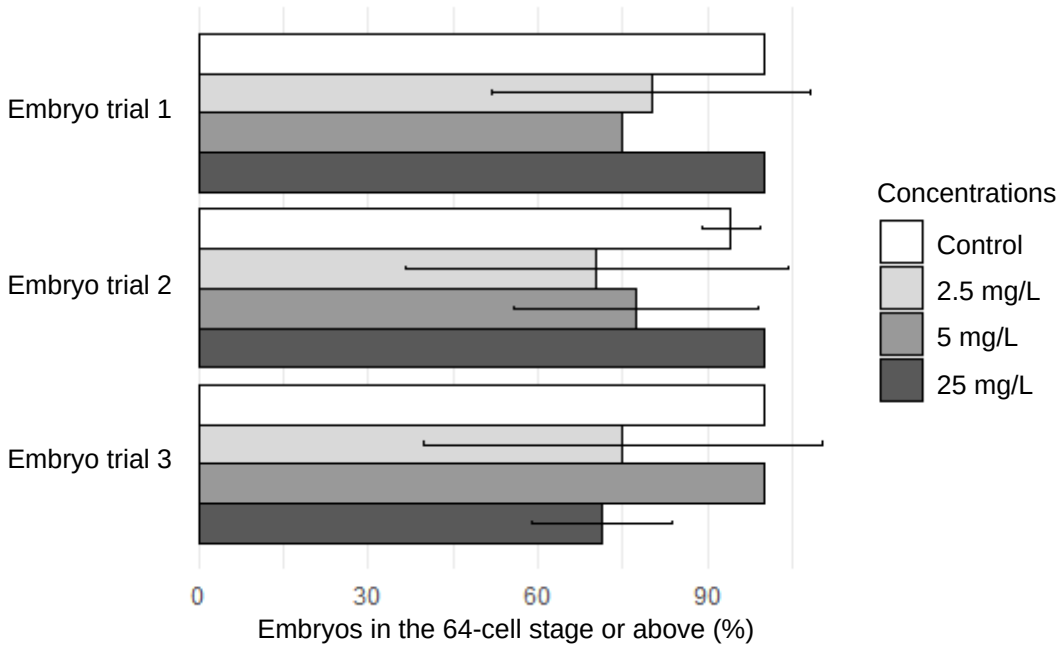

Figure 7. Development stages in the long-term exposure embryo experiments. Proportion of *Lophelia pertusa* embryos in the 64-cell stage or above (mean +/- SD) after 3 days exposure to different concentrations of suspended benthic sediment. See table 1 for more information on the experiments. Replicates in Appendix B2.

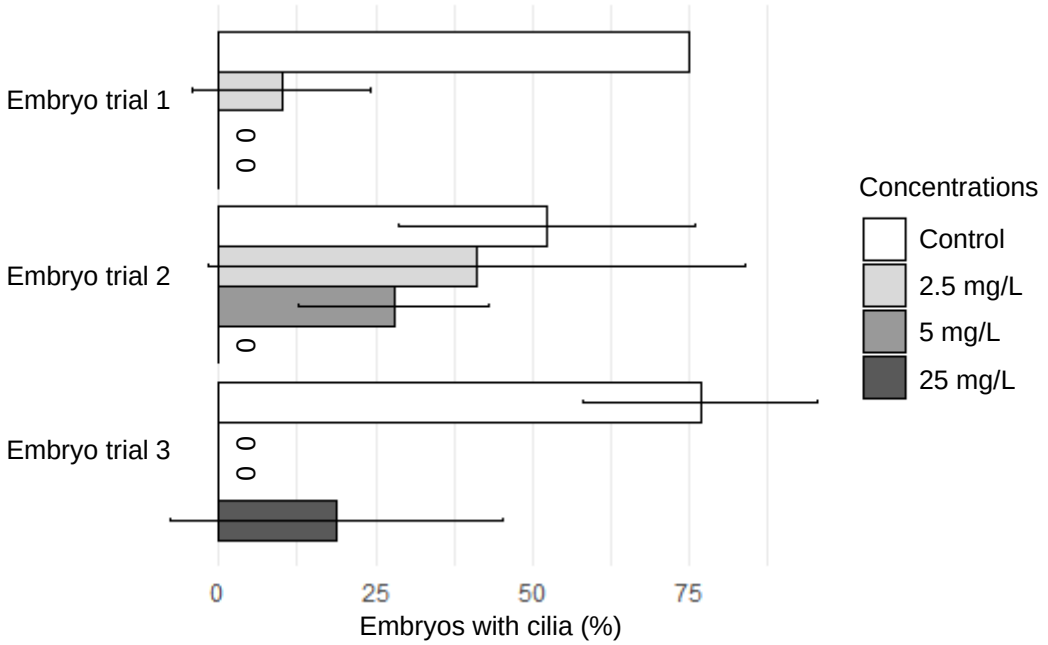

Figure 8. Cilia development in the long-term embryo experiments. Proportion of *Lophelia pertusa* embryos with cilia (mean +/- SD) after 3 days exposure to different concentrations of suspended benthic sediment. Groups with 0% embryos with cilia in all replicates are marked with a zero (0). See table 1 for more information on the experiments. Replicates in Appendix B2.

3.4. Larval swimming speed

The effect of sediment concentration on larval swimming speed varied somewhat between the two experiments performed (Fig. 9). There was a significant difference between the mean swimming speed in the 5-day old larvae, that had been photographed in the sample flasks with sediment, (one-factor ANOVA, $F(3,12) = 3.86$, $p = 0.04$). The swimming speed was significantly higher in the 2.5 mg/L sediment treatment compared to the 25 mg/L sediment treatment (Table 2C). However, none of the sediment treatments groups had a different swimming speed from the control group. There was no difference in swimming speed for the 12-day old larvae, that were photographed in a tank of clean water, (one-factor ANOVA, $F(3,6) = 0.61$, $p = 0.63$), even though they came from the same spawn batch as the 5-day old larvae.

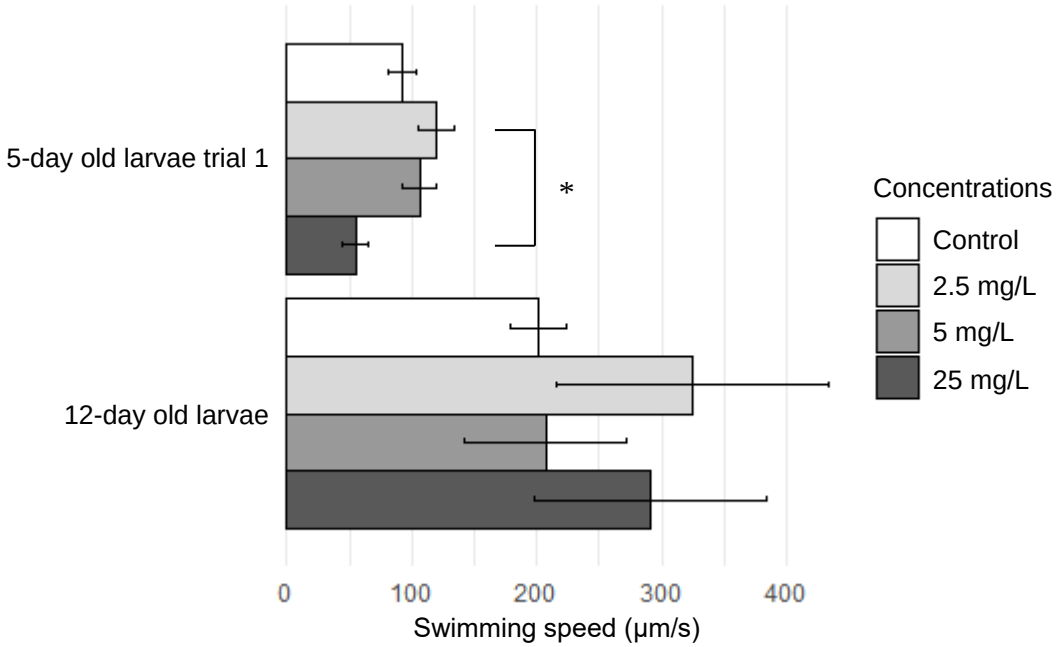

Figure 9. Swimming speed of *Lophelia pertusa* larvae (mean +/- SE) after 3 days exposure to different concentrations of suspended benthic sediment. See table 1 for more information on the experiments. Asterisk (*) for comparison with significance ($p < 0.05$). Replicates in Appendix B3.

4. Discussion

4.1. Embryonic and larval survival

The effect of suspended sediment varied among the experiment trials. If only comparing survival, it appears that a large part of the variation in embryo survival is due to the duration of exposure. The embryos exposed to sediment treatment for one day survived as well as the control group, while those exposed to sediment treatment for three days had much lower survival in higher sediment concentrations. Previous studies using short-term sediment exposure on embryos of tropical coral species also found that the survival was not affected (Gilmour 1999; Ricardo et al. 2016). The embryos in these studies survived sediment concentrations of ~100 mg/L and ~1000 mg/L for 18 and 30 hours respectively. To my knowledge, so far there have been no studies using three days exposure of suspended sediment on coral embryos.

Another important factor is age differences. Among the 3-day exposure experiments on embryos and larvae, there seems to be an age-dependent effect. The embryo survival decreased with increased sediment concentration and the vast majority died in the highest treatment at 25 mg/L. Meanwhile, larvae were usually more tolerant with similar survival rates in each treatment group. A similar age-dependent effect was found in two other studies on *L. pertusa*, which both showed that younger larvae (5 or 8 days old) had higher mortality than older larvae (19-21 days old) at the same concentrations of drill cuttings (Järnegren et al. 2017; 2020). Additionally, a concentration-dependent effect has also been found in previous studies, as each study on larvae of *L. pertusa* reported a decreasing survival in increasing concentrations of drill cuttings (Järnegren et al. 2017; 2020; Larsson et al. 2013).

My results on 5-day old larvae were contrasting: after three days of exposure, one trial showed significantly decreased survival in both 5 mg/L and 25 mg/L of sediment, while the other trial showed tolerance. The trial with a decreased survival also had a significantly lower baseline survival, seen when comparing the control groups between the two trials. Hence, this variation could be an indication of multiple stressors (the other stressor being e.g. infestation by bacteria or parasites), causing a synergistic effect. However, seeing that one of the trials did tolerate 25 mg/L of sediment, it is certainly possible for some 5-day old larvae to survive this range of concentrations.

The oldest larvae from my survival experiments showed tolerance after being exposed to sediment for 3 days, which ended when they were 12 days old. Previous studies on larvae of roughly the same age show vast different responses. Larvae of 23 days age had a 33% survival rate in 25 mg/L of drill cuttings, while the survival rate in the control group was 100% (Larsson et al. 2013). Larvae of 19-21 days age had over 90% survival in drill cutting concentrations up to 160 ppm, which was fairly similar to the survival rate in the control groups, which was over 95% (Järnegren et al. 2017; 2020). These differences between studies can again be explained by the duration of exposure. Both studies

by Järnegren (2017; 2020) used a duration of 24 hours of exposure, which resulted in much higher tolerance than the larvae used by Larsson (2013) after 5 days of exposure. Assuming that drill cuttings and the benthic sediment used in this study are comparable, my results on 9-day old larvae would fit in with previous results. Thus, it would seem like *L. pertusa* larvae over the age of 9 days can tolerate high concentrations of suspended particles for 24 hours or at least 25 mg/L for 3 days, but not for 5 days.

However, comparisons between studies should be done with caution, since the effects of suspended sediment depend on both sediment type, grain size and sediment content (Humphrey et al. 2008). Ideally, future studies should be more extensive, by for example including several sediment types, durations of exposure, and a wider range of concentrations and ages.

4.1.1. Embryo cleavage

In all embryo experiments, small embryos were found in varying sizes. These were likely caused by the cleavage of an embryo into two or more smaller embryos, which has been seen in both *L. pertusa* and other coral species (Heyward and Negri 2012; Larsson et al. 2014). Even a moderate turbulence can cause embryos to fall apart in the 2-, 4- and 8-cell stage and they continue to develop normally into functional larvae in proportionally smaller sizes (Heyward and Negri 2012). The authors also reported that embryos in the 16-cell stage were seen splitting, but without information on further success. My observations on *L. pertusa* suggest that embryo cleavage also can occur in later cell-stages, including in the 64-cell stage. Although, embryos in smaller sizes were typically fragile. This is consistent with the results from a study that split embryos artificially, which found a higher mortality in smaller embryos (Okubo et al. 2017). The high proportions of small embryos in higher concentrations suggest that suspended sediment can trigger the same response as turbulence. If this is true, then it is a new mechanistic pathway explaining the negative effects of suspended sediment, as well as the difference in tolerance between embryo and larvae.

4.2. Embryo development

Although the short-term exposure did not affect embryo survival rate, it significantly affected their cell-stage, while the opposite seemed to be the case for embryos after 3 days of exposure. One possible explanation is that the embryos in the short-term experiment started treatment too early and had not finished fertilizing. Studies on tropical coral species have found that sediment affects fertilization and not embryo development (Erftemeijer et al. 2012; Gilmour 1999; Humphrey et al. 2008). If this is the case for *L. pertusa*, the difference in the short-term experiment could have been caused by the embryos in the high concentration having lower fertilization. It is unknown if the development would still differ when compared to the proportion of fertilized embryos. Hence, further research on development should assess the fertilization rate before the start of exposure. Larger

sample sizes should also be used to include potential embryos in higher cell-stages in the high concentration group.

Nevertheless, it is still possible that the fertilization was not affected and that it occurred previous to the sediment exposure. The exposure on embryos started 6-30 hours after gamete release. The embryos in the short-term experiment were > 6h old, which can be compared to the 6h old embryos in the first trial of the 3-day exposure experiment. These were both younger than the two other trials at 22h and 30h age respectively. Still, the first trial in the 3-day exposure experiment had a similar result to the other long-term trials. Additionally, 6h should be enough time for fertilization, since the beginning of the transition into the 2-cell stage is clear at this time (Larsson et al. 2014). Instead, another explanation to the difference between the experiments is that a significant proportion in the short-term exposure was weakened or simply stopped their development already within 1 day, but survived as oocytes. Then if the treatment were to continue, those oocytes would possibly not have developed further and died. That would have led to lower survival after the longer exposure, but without a change in the cell-stage of embryos in higher concentrations.

Of course, the classification of cell-stages in the 3-day exposure experiment was quite broad, as all embryos in and above the 64-cell stage were pooled together. It is possible that a different result would have appeared with more specific categories, especially since there seemed to be a tendency for lack of cilia in higher concentrations. Although, cilia could also have been removed by parasites that have been found in some of the spawn batches (Strömberg and Larsson 2017). Since not much is known about these parasites, the possibility of synergistic interactions with the sediment should be examined.

4.3. Larval swimming speed

The older larvae were overall faster, which is expected as the development of cilia progresses (Larsson et al. 2014). However, there were no effects of suspended sediment on swimming speed. This was surprising, since embryos were observed with sediment attached to them, and sometimes they were completely encased in it. Attachment of particles are known to restrict the mobility of larvae (Järnegren et al. 2020). If a large proportion of the larvae in my higher concentrations had sediment attached to them, the swimming speed should have been slower in those concentrations.

One explanation to the fairly equal swimming speed is that the larvae had already successfully avoided or removed the sediments. Embryos of tropical coral species can survive while being encased in mucus and sediment, to later emerge by beating with their cilia (Ricardo et al. 2016). These authors also observed that already ciliated larvae were avoiding sediment altogether by releasing mucus and beating their cilia. In *L. pertusa*, around one fourth of the larvae (ages 8 and 21 days) had sediment attached to them, after being exposed to between 18-32 mg/L of drill cuttings for 24 hours (Järnegren et al. 2020). Since my treatment exposure ran for 3 days, more larvae were expected to have

sediment attached to them in the highest concentration, unless drill cuttings attach easier than natural sediments. However, it is also possible that some of the larvae sank and were not tracked, since only those that moved were included. Future studies on swimming speed should compare the effect of these sediment types, while using larger sample sizes and including the larvae that do not move.

4.4. Limitations

The experiments that were conducted might have suffered from an experiment-time effect. When comparing different trials of the embryo and 5-day old larvae experiment, it appears as the trials performed later had a stronger effect of the sediment (Fig. 10). Although the embryo experiments were started at different hours of age, the age does not seem to correlate with the pattern of increased sediment sensitivity. One possible cause for an experiment-time effect could have been changes in the sediment during the two months it was used, e.g., increasing build-up of harmful biproducts from an anoxic environment. Moreover, reusing or washing the sample bottles might have affected the surface treatment, perhaps causing sediment lumps to increase with time. Nevertheless, an experiment-time effect could have created the appearance of an age-dependent effect, since most of the larvae experiments happened to be conducted earlier than the embryo experiments. If such was the case, any differences between sediment treatments cannot be attributed to the isolated effect of sediments, but instead, to an unknown stressor(s) in addition to the sediment. However, the lack of a difference between treatments does indicate tolerance to all stressors, including to sediment. Hence, larvae can indeed tolerate sediment of at least 25 mg/L for at least 3 days. To confirm the sensitivity of embryos, experiments should be repeated with an adjusted study design to account for the experiment-time effect.

A batch effect was also found. There were significant differences in survival rate when only comparing the control groups from different trials of experiments on embryos/larvae of the same age. The difference between embryo spawn batches do not seem to correlate with the variation in age. Instead, the variations between embryo trials and between larvae trials may be due to variations in overall health, occurrence of parasites, genotype, or unknown environmental factors. It is unclear if the batch effect only affects the baseline survival, or if it also leads to synergistic effects together with the sediment treatments. In the latter case, it could potentially mean that there was no effect of experiment-time, and that the variations between trials were only affected by multiple stressors. For example, it might be possible for larvae to survive 3 days exposure to suspended sediment, or to some extent survive parasites alone, but not both stressors simultaneously. Future studies should try to define and test each stressor separately, as well as their combined effect.

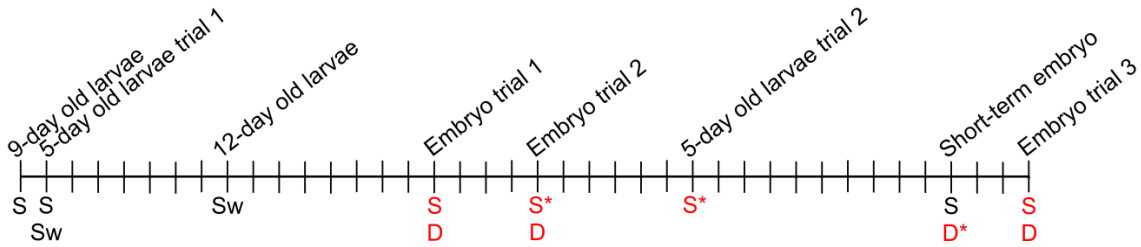

Figure 10. Timescale over 40 days indicating when each experiment started relative to each other. Type of experiments under the scale (S = survival, Sw = swimming speed, D = development). Red color means there was a trend ($p < 0.1$) between control and at least one of the concentrations, asterisk means the difference was significant ($p < 0.05$). The first two embryo trials showed similar sensitivity in the 2.5 mg/L and 5 mg/L treatment, while trial 3 had a much stronger response (Fig. 4). The first embryo trial was much younger than the latter two, suggesting that this pattern is not explained by their age. The first trial of 5-day old larvae saw no difference between concentrations, while the second trial had a significant difference.

4.5. Implications

The results from this study show a decrease in embryo survival, both as a direct response to suspended sediment and indirectly through embryo cleavage. If embryos or larvae of *L. pertusa* have a lower survival rate due to the suspended sediment caused by trawling and drilling, it would clearly have a negative impact for the rejuvenation of the species. In addition, non-lethal effects such as inhibition of the development or loss of buoyancy due to attached sediment can have an impact on the dispersal (Hilario et al. 2015). The embryos development rate, buoyancy and swimming speed can all affect vertical migration. Since the velocity of currents depends on depth, the ability to migrate vertically will in turn affect the dispersal patterns and connectivity of metapopulations, and thereby the genetic diversity (Hilario et al. 2015). My results indicate that embryos might have a decreased ability to migrate vertically, but that the larvae had an unaffected swimming speed. But even if the larvae can recover from the effects of suspended sediment, a temporary decrease in vertical migration could still affect connectivity. Maintaining high connectivity can make the population more resilient to future disturbances and is considered a priority in conservation and the establishment of MPAs (Hughes et al. 2008; McLeod et al. 2009).

In Norwegian waters, both reefs and trawling activities are widespread (Eigaard et al. 2017; Fossa et al. 2002). Since the extent of the impact depends on vicinity and duration of trawling, it would be beneficial if no-trawl zones extended a bit outside of the reefs. Even then, it would be wise to spread the trawling events in time and space. The highest priority is to reduce trawling during the spawning season. Although the larvae seemed tolerant, that did not appear to be the case for embryos. Additionally, there are still knowledge gaps regarding other life stages of *L. pertusa*. Further research should focus on the success of fertilization and larvae settlement during sediment exposure.

4.6. Conclusion

The effect of suspended sediment on the young life-stages of *Lophelia pertusa* was found to depend on the age of embryos and larvae, the duration of sediment exposure, as well as the concentration of sediment. Larvae of *L. pertusa* were able to withstand 25 mg/L of suspended sediment for three days without any detectable effect on their swimming speed. Embryos appeared to survive the same concentrations for one day, although with adverse consequences to either their development or fertilization. Embryos exposed to three days of sediment had a lower survival, often lacked cilia, and commonly responded with cleavage into multiple smaller embryos. Further research should strive to confirm these results, and to study the impact on other life-stages of *L. pertusa*. In the meantime, conservation efforts should aim to minimize the concentration of suspended sediment in *Lophelia* reefs, in order to protect the long-term survival of the reefs themselves, as well as the inhabitants depending on them.

Acknowledgement

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Appendix

Appendix A: Popular science summary

Raising coral babies in filthy water

Far away from the tropics, there are coral reefs thriving in cold water all around the world. Hidden in the deep sea they are rarely visited by humans, but often encounter traces of our activities – in the form of a threat.

The life of Lophelia

The most common species of cold-water corals, *Lophelia pertusa* is usually found at depths between 100-4000 m, in temperatures as cold as 6°C. It is a crucial species for the existence of many deep-sea coral reefs. Their life begins with a spawning event, when eggs and sperm is ejected into the open water and form embryos. The embryos follow the current as they develop into larvae and start to search for a spot to settle at around 3 weeks of age. After settling, they slowly grow into a hard, tree-like structure. During their long lifespan, they can settle on a barren ocean floor and cover it with complex structures. These reefs are home to thousands of species and once found, they often receive the status as a protected area.

Threats

Protected areas prohibit activities that cause physical harm to the corals, such as bottom-trawling and drilling. However, there is concern that such activities could have negative impact to corals even when performed outside of the reef. Bottom-trawling and drilling cause particles from the ocean bottom, the sediment, to mix with the water and become suspended. The sediment concentration rises to unnatural levels and can be carried with the current for several kilometers. This causes problems for corals, especially in their early life-stages. In tropical coral species, suspended sediment inhibits fertilization and increases the mortality of larvae. The response to suspended sediment depends on coral species, but researchers rarely focus on cold-water corals.

Current research

At the Tjärnö Marine Laboratory, the coral team is working with *Lophelia* fragments which they have collected with a remotely operated underwater vehicle. The adult corals are kept in aquariums filled with cold water. After each spawning, they collect the water containing eggs and sperm for later use in the experiments. I was able to conduct a series of experiments on both embryos and larvae, to find out how they respond to suspended sediment. I measured differences in survival, development, and swimming speed after exposing them to either clean water, or one of three sediment concentrations.

I found that the embryos and larvae responded differently to sediment exposure. Most of the larvae survived high concentrations of sediment for 3 days and were equally fast at swimming as the larvae in the clean water. However, after 3 days in sediment, embryos had a decreased survival rate in the higher sediment concentrations. They would often fall apart into two or more smaller embryos, which would easily dissolve and die. Other embryos were only exposed to sediment for one day and seemed to survive, but with consequences to their development. If the survival and development of embryos are affected in the wild, it could have consequences for the long-term survival of the species. Hopefully, trawling can be avoided around *Lophelia* reefs, especially during the spawning season. This could help us protect the embryos and larvae of cold-water corals as well as the numerous species dependent on them!

Appendix B: Tables of replicates and sample sizes

Table B1. Sample sizes and replicates used for assessments on survival and proportion of small embryos of *Lophelia pertusa*. Replicates are number of sample flasks per treatment group. Sample size is the number of embryos/larvae per sample flask before sediment exposure.

Experiments	Replicates	Sample size
Short-term exposure	3	36
Embryo trial 1	4-5	50
Embryo trial 2	4	37-50
Embryo trial 3	3	52
5-day old larvae trial 1	5	52
5-day old larvae trial 2	3	26-30
9-day old larvae	3	13-14

Table B2. Sample sizes and replicates used for assessments on development in *Lophelia pertusa* embryos. Start total refers to individuals per sample flask before sediment exposure. The sample size is the number of embryos per sample flask that were analyzed by observing in microscope, after the exposure to sediment. Replicates are number of sample flasks per treatment group that were used for analysis. The samples are subsamples of survivors from the survival assessments.

		Sample sizes			
Experiments	**Start total**	**Control**	**2.5 mg/L**	**5 mg/L**	**25 mg/L**
Short-term exposure	36	12, 10, 12	-	-	12, 7, 16
Embryo trial 1	50	4	1, 5	4	3
Embryo trial 2	37-50	13, 10, 10	10, 9, 9	8, 7, 7	2
Embryo trial 3	52	10, 11	4, 2	4	8, 5

Table B3. Sample sizes and replicates used for assessments of swimming speed in *Lophelia pertusa* larvae. Start total refers to individuals per sample flask before sediment exposure. The sample size is the number of larvae per sample flask that were tracked in ImageJ, after the exposure to sediment. Replicates are the number of sample flasks per treatment group that were used for analysis.

		Sample sizes			
Experiments	**Start total**	**Control**	**2.5 mg/L**	**5 mg/L**	**25 mg/L**
5-day old larvae	52	2, 6, 5, 4	6, 1, 1, 4, 2	4, 6, 4, 2	2, 6, 2
12-day old larvae	50	3, 4, 1	1, 5, 1	1, 3	1, 2