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Fish farm effluents cause metabolic depression, reducing energy stores and growth in the reef-forming coral *Lophelia pertusa*

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ABSTRACT: Cold-water corals (CWCs) have come under increasing pressure from human activities over the last decades. Of particular concern in Norway is the potential impact of open net pen aquaculture on CWC reefs formed by *Lophelia pertusa,* a threatened and declining habitat. We conducted a 1 yr *in situ* transplantation experiment and corresponding field measurements of 2 reefs located close to fish farms to elucidate the impacts of particulate organic waste released from the farms on coral colonies. Our study provided new evidence of negative impacts of organic effluents on *L. pertusa* ecophysiology. After 1 yr, both naturally occurring and transplanted corals, at distances ranging from 250 m to 1 km downstream of what would be regarded as an average-sized Norwegian fish farm, exhibited depressed metabolic rates compared to corals outside the main depositional footprint of the farms. The metabolic depression impeded energy acquisition, reducing growth and energy reserves by up to 70 and 50%, respectively. No clear threshold for significant biological impact could be detected along a distance gradient. Instead, a gradual decrease in metabolic rates, growth and lipid reserves occurred with increasing modelled sedimentation rate of organic waste from the farm. The strong statistical correlation between oxygen consumption, growth, energy stores and sedimentation rates implies that predictions of the short-term impact of aquaculture effluents on *L. pertusa* ecophysiology and CWC reef development may be achievable in the future. This would significantly improve the ability of management to make informed decisions on the licensing of new farms near CWC reefs.

KEY WORDS: Cold-water corals · Vulnerable habitats · Human impact · Aquaculture · *Desmophyllum pertusum*

1. INTRODUCTION

Coastal areas in Norway are increasingly subjected to stress from human activities such as fishing, aquaculture and mining, all of which have some degree of impact on the surrounding marine ecosystem (Fosså et al. 2002, Ramirez-Llodra et al. 2015, Taranger et al. 2015). With one of the most complex marine landscapes in the North-East Atlantic, Norwegian coastal areas harbour patches of highly diverse and productive ecosystems such as cold-water coral (CWC) reefs, sponge gardens, maerl beds and kelp forests that provide important ecosystem functions in terms of nutrient and organic matter cycling, primary production, carbon storage, and as nursery grounds and important habitat for many commercially harvested species (Nelson 2009, Freiwald et al. 2012, Cathalot et al. 2015, Araújo et al. 2016), requiring specific management attention. Of particular importance in Norwegian coastal waters is the increasing conflict between open net pen salmon aquaculture, which is estimated to grow further with

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the continued expansion of the blue economy, and the sensitive, threatened and declining reefs formed by the CWC *Lophelia pertusa* (i.e. *Desmophyllum pertusum*). Norway's long and jagged coastline provides excellent conditions for the growth and development of *L. pertusa* reefs as well as ideal conditions for salmon production in open net pens. Conservation, adaptive use and management are key to achieving sustainability and to ensuring a continued provision of economic and environmental benefits of these ecosystems. However, lack of knowledge on factors that can significantly influence reef health limits the implementation of correct management measures to ensure the protection of these nationally iconic reefs.

More than 6000 *L. pertusa* reefs have been registered on the Norwegian continental shelf and inside the deep fjords, representing roughly 25% of the known global occurrence of this species (Järnegren & Kutti 2014). Here, these reefs form isolated diversity and productivity hotspots at depths between 100 and 600 m (Freiwald et al. 2012, Cathalot et al. 2015) which are entirely dependent on organic material produced in the sunlit surface layer of the ocean (Duineveld et al. 2004, Kiriakoulakis et al. 2005, van Oevelen et al. 2018). As the sedimentation of organic particles from the surface occurs mainly in connection with the settling of the spring and autumn plankton blooms, CWC reefs commonly occur in hydrodynamically complex areas where the delivery of food particles to the seabed is elevated, either through the acceleration of bottom currents increasing the horizontal particle supply or through processes such as tidal pumping and Ekman drainage increasing the vertical particle supply (Thiem et al. 2006, Davies et al. 2009). In fjords, thriving reefs are often found where water is forced to accelerate, such as on morainic sills (Mortensen & Fosså 2006, Rüggeberg et al. 2011) and at locations with vertical walls (Järnegren & Kutti 2014). The same areas are also favoured by the aquaculture industry because high flow is critical in bringing well-oxygenated water to the caged fish while at the same time removing waste.

Close to 1000 salmonid farms were in operation in Norway by 2019, producing approximately 1.1 million t of fish (Grefsrud et al. 2021). The production takes place in open net cages; hence, waste feed and the faeces produced by the fish are released into the surrounding ecosystem. It is estimated that an average-sized farm will release about 1.3 t of particulate organic matter (POM) every day at peak production (Brooks & Mahnken 2003), which accounts for a total of 500 000 to 700 000 t of POM release annually from all Norwegian salmon farms combined (Grefsrud et al. 2018). Organic waste released from the caged fish is dispersed from a few hundred up to 1000 m away from the farms, elevating the sedimentation of POM at the seabed near the farms 8 to 20-fold normal background sedimentation (Kutti et al. 2007a, Keeley et al. 2019). Furthermore, therapeutants and heavy metals originating from the feed or antifoulants may be released into the surrounding environment and accumulate in the bottom sediments (Samuelsen 2016, Grefsrud et al. 2021). In 2016, more than 24 fish farms were known to be located within 2 km of a CWC reef (Husa et al. 2016). Since then, several new farms have been established. As deep coastal areas are poorly mapped, it is likely that the actual number of overlaps is much larger than 24 and increasing.

Impacts of farm derived POM on communities of soft sediment dwelling benthic fauna include subtle changes in species composition and total abundance away from the farm, where sedimentation rates of POM are close to background rates. Close to the farm, where sedimentation rates of POM are much higher, complete shifts in species composition and increased abundances of a few opportunistic species occur (Kutti et al. 2007b, 2008, Bannister et al. 2014, Keeley et al. 2019). There is, however, only sparse knowledge on how hard-bottom benthic communities respond to elevated sedimentation rates of POM. In particular, information on how long-lived sessile fauna, such as CWCs, respond to increased organic loading is lacking. This is a pressing concern because CWCs are dominant components of the benthic environment in Norwegian waters (www.mareano.no), are listed as sensitive and declining habitats by the OSPAR commission (www.ospar.org) and are being increasingly affected by aquaculture activity. Re-establishing dead or severely damaged reefs will take centuries, if not longer, because CWCs have slow growth rates (Maier et al. 2020) and very low recruitment rates (Lacharité & Metaxas 2013, Doughty et al. 2014).

The objective of this study was to generate new knowledge on the resilience of *L. pertusa* reefs to effluents from open net pen aquaculture and to quantify the impact of the sedimentation of organic waste from fish farms on *L. pertusa* ecophysiology. Such data are needed for management to make informed decisions on requests to establish new farms and requests to increase standing biomass at existing farms located near CWC reefs. Due to the patchy natural distribution of CWC reefs, *L. pertusa* fragments were transplanted to 6 sites along a gradient away from 1 fish farm (250–2000 m) and exposed to organic farm waste *in situ* for 1 yr. At 2 other sites, natural populations of

L. pertusa growing 300 and 500 m away from fish farms were sampled. The approach of combining an *in situ* transplantation experiment and field examinations of natural reefs was selected because high-resolution spatial gradients of CWC reefs located at different distances away from farms have, to our knowledge, not been found. Collectively, the transplantation study and the examination of natural reefs were expected to give a comprehensive understanding of the impacts of fish farm effluents on *L. pertusa* colonies. Physical and biological health indicators (i.e. respiration and ammonia excretion rates, lysosomal membrane destabilization rates, lipid stores and growth) were measured and correlated with modelled sedimentation of particulate organic farm waste at the collection sites.

2. MATERIALS AND METHODS

2.1. Study sites

This study was undertaken in an archipelago of central Norway (Fig. 1), a region characterized by a rugged undersea landscape with deep and steep-flanked fjords highly suitable for CWC growth. Moreover, the area is intensively used for aquaculture production in open sea cages. We studied 3 averaged-sized fish farms, each located in a different fjord, within 40 km of each other (Fig. 1). At the first farm in Julsundet (JS), located in an area with relatively even bottom topography, we deployed 6 benthic landers for the *in situ* coral transplantation experiment examining the 1 yr of exposure to farm effluent at a high spatial resolution. The other 2 farms, located in Midtfjorden (MF) and Sunndalsfjorden (SF), were located on steep flanks, each with 1 small CWC reef growing close to the open net cages.

2.2. *In situ* transplantation experiment

Colonies of *Lophelia pertusa* were collected at 150 m depth on the coastal

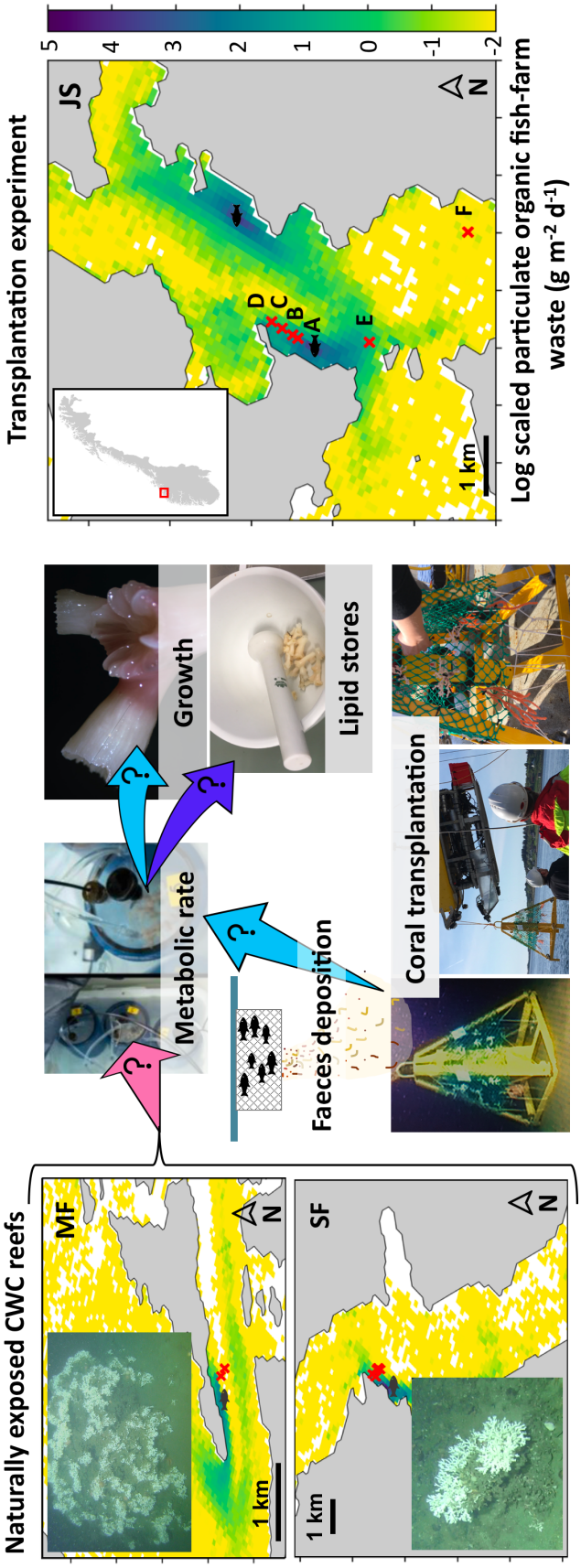

Fig. 1. Study sites Midtfjorden (MF), Sunndalsfjorden (SF) and Julsundet (JS) in mid-Norway. Left panels: Modelled average sedimentation rates at MF and SF (during the same time period as the JS experiment) at 2 reefs naturally exposed to the deposition of faeces from nearby aquaculture facilities. Centre panel: Workflow of the transplantation and field collections and the eco-physiological measurements performed to assess the effects of the deposition of organic waste from fish farms on the cold-water coral (CWC) *Lophelia pertusa*. Right panel: Modelled average sedimentation rates at JS during the 1 yr transplantation experiment and localization of the 6 transplantation rigs (A–F). : fish farm; ×: coral collection site. Inset right panel, □: study area

CWC reef Kjerringrevet (62° 46.3′ N, 06° 56.0′ E) on Institute of Marine Research (IMR) research cruise 2018612 with the RV 'Kristine Bonnevie' (10–15 May 2018) using the remotely operated vehicle (ROV) 'Aglantha'. Onboard coral colonies were maintained (for 48–72 h) in a 3000 l covered fish tank filled with deep water collected from 100 m depth at the same site, replacing 50% of the water every 12 h. Baseline measurements of the corals' respiration and ammonia excretion were taken by incubating 5 fragments (approximately 15–20 polyps in size) in 1000 ml cylindrical polycarbonate respiration chambers. Five other fragments were frozen (–20°C) for later determination of lipid content. All these measurements were carried out following the procedures described in Section 2.4.

Fragments for 6 transplantation units were prepared by cutting *L. pertusa* colonies (n = 40) into pieces of 20 to 40 polyps. A total of 60 fragments were stained for 48 h in 4 separate 40 l tanks containing deep water which had been mixed with alizarin red (A5533, Sigma Aldrich) to form a concentration of 10 mg l^{-1}, following Brooke & Young (2009), in order to record linear growth 72 similar-sized nubbins were left unstained and intended for physiological measurements and cellular and biogeochemical analysis after recovery. For each experimental site, the pyramid was prepared just before deployment by attaching 8 stained and 12 unstained coral nubbins (originating from different mother colonies) evenly over a 5 × 5 cm nylon mesh covering the 4 sides of an 80 cm tall aluminium frame pyramid (see Fig. 1). Each pyramid was fitted with a cNODE transponder (Kongsberg Maritime) and 10 kg of lead weight at the bottom of the frame. Using the ROV, pyramids were deployed 250, 500, 750 and 1000 m north and 1000 and 2000 m south of the farm in JS (Sites JS A–F; Table 1).

The transplanted *L. pertusa* fragments were retrieved after 13 mo during a research cruise with the RV 'Kristine Bonnevie' (13–21 June 2019, IMR cruise 2019619) using the ROV. Each transplantation unit was retrieved, and samples were all processed in the exact same manner, with 2 units retrieved each day, 1 in the morning and 1 in the afternoon. Upon retrieval, coral nubbins were cut off the pyramids and immediately transferred to a 1000 l fish tank containing deep water (8°C) collected from 100 m depth at the retrieval site. Six unstained fragments were transferred to a controlled-temperature water bath (8°C), where corals were incubated in sealed chambers to measure their respiration and ammonia excretion rates. Another 6 fragments were subsampled and stored at –20°C for later determination of lipid content. Five small fragments of 2 to 4 polys each were immediately processed for measurements of lysosomal membrane stability (LMS). The stained coral nubbins were frozen (–20°C) and thawed one by one for linear growth determinations in the laboratory onboard. The chosen endpoints (i.e. LMS, respiration, ammonia excretion, lipid stores and growth) provide information on cellular homeostasis, energy acquisition, utilization and allocation in coral colonies and give important clues of the overall health of the colonies. They have proven to be sensitive indicators of a range of environmental stressors including hydrodynamics, food availability, temperature and exposure to suspended sediments (Larsson et al. 2013, Edge et al. 2016, Jacobson et al. 2016, Lartaud et al. 2017, Baussant et al. 2018, Scanes et al. 2018).

2.3. Natural reefs — transplantation control

In June 2019, 5 small colonies of *L. pertusa* were collected at 190 m depth on a patch reef located 300 m away from the farm in MF using an ROV (Table 1). This salmon farm was established in 2011 and had a maximum allowable biomass of 3000 t. Five other

Table 1. Distance and cardinal direction from the closest fish farm to the collected corals as well as geographical position, depth, modelled sedimentation rate of particulate organic matter (POM) and mean and maximum bottom current speed (from the NorFjords160 model). JS: Julsundet; MF: Midtfjorden; SF: Sunndalsfjorden; na: not analysed

Site	Distance (m) and cardinal direction to farm	Lat. (°N)	Long. (°E)	Depth (m)	POM deposition (g m^{-2} d^{-1})	Mean bottom flow (cm s^{-1})	Max. bottom flow (cm s^{-1})
JS A	250, N	62.787	6.933	160	6.64	0.09	0.47
JS B	500, N	62.789	6.935	164	3.60	0.09	0.48
JS C	750, N	62.791	6.938	178	2.69	0.10	0.53
JS D	1000, N	62.793	6.940	180	1.64	0.11	0.60
JS E	1000, S	62.770	6.932	160	1.74	0.13	0.65
JS F	2000, SE	62.745	6.980	160	0.11	0.07	0.42
MF	300, E	62.640	6.563	190	0.05	na	na
SF	700, NE	62.913	7.120	190	1.73	na	na

small *L. pertusa* colonies were collected at 190 m depth on a reef located 500 m away from the farm in SF (Table 1). This farm was established in 1995 and had a maximum allowable biomass of 2300 t. Onboard coral colonies were maintained until processing in a large fish tank (1000 l) filled with deep water collected from 100 m depth at the same site. From each colony, 1 fragment (approximately 15 polyps in size) was incubated to measure respiration and ammonia excretion rates. Another fragment from each colony was frozen (–20°C) for later determination of lipid content. A small piece (2–4 polyps) was analysed immediately for LMS. All measurements were done following the procedures described below.

2.4. Sample analyses

2.4.1. Cellular stress

Lysosomal integrity is essential for maintaining cellular homeostasis. Hence, assays determining the integrity, or stability, of the lysosomal membranes within cells have successfully been used as indicators or proxies for cellular health and early indicators of stress in organisms exposed to, for example, elevated temperature, enhanced levels of suspended sediments and chemical pollutants (see e.g. Brown et al. 2004, Edge et al. 2016, Scanes et al. 2018). Here, LMS was assessed in corals growing along an enrichment gradient away from 3 fish farms using the neutral red retention assay, following methods adjusted from Ringwood et al. (2003), Edge et al. (2016) and Scanes et al. (2018). Briefly, the coral piece was crushed in a mortar and mixed with 1 ml Ca^{2+}- and Mg^{2+}-free seawater (CMFS). The sample was then agitated for 30 min on ice. Subsequently, the coral homogenate was filtered through an 80 µm plankton mesh into a 2 ml microcentrifuge tube, and the clear fluid was centrifuged at 300 × *g* for 5 min. The supernatant was discarded and the pellet was resuspended in 1 ml CMFS and centrifuged again at 300 × *g* for 5 min, after which the supernatant was removed. After agitation and an addition of 50 µl neutral red dye (N4638, Sigma-Aldrich), the remaining cell suspension was incubated at room temperature in the dark for 1 h. The retention of neutral red into the lysosomes was then examined using light microscopy (40× magnification). A total of 50 cells were scored as stable, i.e. cells with the neutral red dye retained in the lysosomes, or unstable, i.e. cells with neutral red leaking into the cytoplasm. The percent destabilization was calculated for each fragment.

2.4.2. Respiration rate and ammonia excretion

In the metabolic process where organisms break down complex organic molecules to acquire energy, oxygen is consumed, and nitrogen is released as a byproduct. Oxygen consumption is governed by the organisms' energy demand, and the amount of nitrogen excreted can provide information on which substrate is utilized in the catabolism (Mayzaud & Conover 1988); together, these measurements provide important information on the variability in food availability and utilization both in time and in space (see e.g. Morley et al. 2016, Maier et al. 2020, Laroche et al. 2022). Here, respiration and ammonia excretion measurements were performed in 1000 ml cylindrical polycarbonate incubation chambers fitted with submersible micropumps operating at a speed of 100 ml min^{-1} to ensure non-static conditions. The chambers were used in flushing mode until most of the polyps had their tentacles extended, after which the chambers were sealed and incubations commenced. Oxygen concentration in the incubation chambers was measured continuously using optical probes (OXY-4 SMA®, PreSens), and the incubations lasted until 70 to 80% oxygen saturation had been reached, about 2 h. Water temperature in the chambers was kept constant by submerging the chambers in a 1000 l fish tank connected to a water cooler (AquaMedic Titan 1500). For the duration of the incubations, chambers were kept in dark conditions. Before and after each incubation, 20 ml water samples were collected from each chamber and stored frozen for fluorometric determination of ammonium by direct segmented flow analysis (Alpkem Flow Solution IV autoanalyser; Kérouel & Aminot 1997, Holmes et al. 1999) in the chemistry lab of IMR, Bergen. Water volume of the chamber was subsequently measured. Coral nubbins were dried at 65°C for 7 d to determine the dry weight (DW) and afterwards combusted at 480°C for 5 h to measure the ash-free dry weight (AFDW). Changes in oxygen and ammonia concentrations in empty seawater control chambers were used to measure the oxygen and ammonia consumption by microorganisms in the seawater.

The respiration rate of the coral fragments was calculated as the regression coefficient of the linear decrease in oxygen concentration in the seawater of the chambers. The respiration rate (expressed as oxygen consumption) was calculated using the auto_rate function of the package *respR* (Harianto et al. 2019), R v.3.5.2 (R Core Team 2020), as follows: Respiration rate (µmol O_2 g $AFDW^{-1}$ h^{-1}) = ($\Delta O_2 \times V$) / ($\Delta t \times$ AFDW), where Δt is the duration between the first

and the last point of the linear regression (h), ΔO_2 is the difference in oxygen concentrations between the first and the last point of the linear regression (µmol O_2 l^{-1}), V is the volume of seawater in the chamber (l), and AFDW is the ash-free dry weight of incubated coral fragments (g). The ammonia excretion rate (E) was calculated as follows: $E = (\Delta NH_4^+ \times V) / (\Delta t \times$ AFDW), where Δt is the time between the first and last water samples (h), and ΔNH_4^+ is the difference in ammonia concentrations at the start and end of the incubations (µmol N g AFDW^{-1} h^{-1}). Changes in oxygen and ammonia concentration in control chambers were used for background corrections of respiration and excretion rates.

2.4.3. Growth rates

From each site to which corals had been transplanted, the linear growth was measured for 7 to 9 fragments with 20 to 40 polyps each as the linear extension of the polyp skeleton from the red band stained with alizarin to the outer edge of the coral calyx using digital callipers (Brooke & Young 2009). Polyps were categorized into (1) old terminal polyps, i.e. terminal polyps that had been stained before deployment; (2) young terminal polyps, i.e. new polyps that had started to develop on the old terminal polyps before deployment and that had been properly stained; and (3) new polyps, i.e. non-stained polyps that had started to form during deployment. Annual linear extension (mm polyp^{-1} yr^{-1}) was calculated for the young polyps of each fragment. For each *L. pertusa* fragment, the rate of formation of new polyps, i.e. annual budding rate (from May 2018 to June 2019), was subsequently calculated using the following formula: Number of new polyps / number of terminal calices in the fragment × 100. Number of dead polyps was registered for all fragments, and an annual polyp mortality rate was estimated as follows: Number of dead polyps / total number of polyps of the fragment × 100.

2.4.4. Tissue energy reserves

Lipids were extracted from 2 to 4 g of frozen coral using a modified Folch method (Folch et al. 1957, Meier et al. 2006). Briefly, samples were crushed using a mortar, weighed and added to 7 ml of chloroform:methanol mixture (2:1, v/v). After sonication (Branson 5200) for 5 min, the mixture was filtered into a 25 ml glass vial, and the solid residue on the filter was rinsed with the same mixture of chloroform: methanol 2 times. Then, 4.5 ml of 0.88% KCl was added to the combined filtrates of 18 ml and shaken vigorously. The sample was centrifuged at 2000 rpm (417 × g) at 16°C for 5 min. The supernatant was removed. $NaSO_4$ powder (3–4 g) was added to the mixture to remove the remainder of the non-lipid layer, and the mixture was filtered again. The remaining solvent was evaporated using nitrogen gas. The lipid content was determined gravimetrically using a 4-figure balance (Mettler AE 163).

2.5. Dispersal and sedimentation of particulate organic waste

The dispersion of organic matter from the farms was modelled using the Lagrangian dispersion model LADiM (Ådlandsvik & Sundby 1994). Each particle was randomly assigned a value for vertical sinking velocity from an empirical distribution for salmonid faeces in accordance with Bannister et al. (2016). Fifty particles were released every hour, from 10 m depth, and the dispersion was calculated using a 4th order Kunge-Rutta scheme solving the Lagrangian equation of motion with a timestep of 20 s. The currents used to transport the particles were obtained from NorFjords160, a downscaled version of the Regional Ocean Modeling System (www.myroms.org; Shchepetkin & McWilliams 2005, Haidvogel et al. 2008, Albretsen et al. 2011), with a 160 × 160 m horizontal resolution. The model was set up with 35 sigma layers in the vertical, and the currents used to calculate the particle transport were stored every hour. No resuspension was implemented in the model; it simply calculated the initial footprint of organic particulate matter. The daily average sedimentation rate of POM (g m^{-2} d^{-1}) at each sampling location was calculated using a daily feeding rate of 0.59% of the average biomass (based on Kutti et al. 2007a, Haugland 2019) for the time period for which the experiment lasted, assuming no feed spill and that 13% of the feed was released as faecal material (Brooks & Mahnken 2003). For coral sampling sites that also had another farm located within a 2 km radius, POM sedimentation rates were estimated, releasing a relevant number of particles from these farms also, based on their biomass. Environmental observations at the site at the time of the experiment were not available, but the model has previously been shown to reproduce environmental variables comparable to observations. The vertical gradient of temperature and salinity is slightly underestimated, but the deviation

is typically less than 1°C and 1 unit in salinity from observations (Asplin et al. 2020). The simulated currents coincide well with the observations 64 to 87% of the time (Dalsøren et al. 2020).

2.6. Statistical analysis

To test for differences in respiration rates, ammonia excretion, lipid concentration, LMS and growth of corals transplanted to different distances away from a fish farm, 1-way ANOVAs with Tukey's HSD post hoc analyses were used. Before analysis, normality was verified using the Shapiro-Wilk test, and homogeneity of variances was verified using Levene's test (respiration, lipid and growth data). Pearson's correlation tests were used on the full dataset to assess correlations between modelled sedimentation of organic waste from the 3 fish farms and the oxygen consumption rates of the corals as well as between oxygen consumption rates and lipid stores (all corals) and growth (only transplanted corals). All analyses were run using R v.3.5.2 (R Core Team 2020).

3. RESULTS

3.1. Respiration rates

Significant differences in *Lophelia pertusa* respiration rates were found among the 6 transplantation sites (1-way ANOVA: $F(5,30) = 8.77$, $p < 0.01$) (Fig. 2), with a trend of decreasing oxygen consumption when moving closer to the farm. Oxygen consumption was significantly depressed in coral fragments that had been growing 250, 500 and 750 m north of the farm, when compared to the reference site 2000 m south of the farm, and significantly depressed in fragments growing 250 and 750 m north of the farm and the reference site 1000 m south of the farm (Tukey's HSD test, $p < 0.05$). Mean (±SE) oxygen consumption at the point closest to the farm was 3.4 ± 0.6 µmol O_2 g AFDW^{-1} h^{-1}, which was 62% lower than the mean oxygen consumption of corals growing at the 2 reference sites where oxygen consumption was 9.0 ± 0.8 µmol O_2 g AFDW^{-1} h^{-1}. In comparison, oxygen consumption was 10.9 ± 0.46 µmol O_2 g AFDW^{-1} h^{-1} in freshly collected coral fragments from Kjerringrevet in May 2018 before the transplantation experiment commenced. In coral fragments from the natural reef 300 m away from the fish farm in MF and used as a transplant control, mean (±SE) oxygen consumption was 7.3 ± 0.9 µmol O_2 g AFDW^{-1} h^{-1}; 500 m away from the fish farm in SF, oxygen consumption was 5.7 ± 0.7 µmol O_2 g AFDW^{-1} h^{-1}.

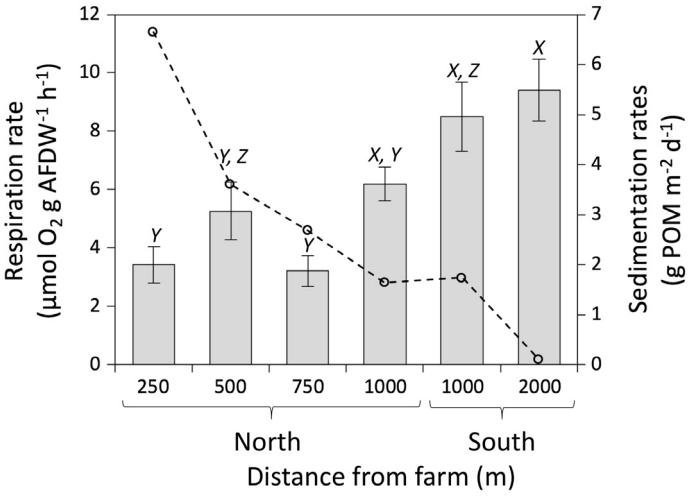

Fig. 2. Respiration rates (mean ± SE) of *Lophelia pertusa* coral fragments after 1 yr of transplantation from Kjerringrevet (a nearby cold-water coral reef) to a gradient away from a salmon fish farm. n = 6 for all sites. Dashed line shows the modelled average daily sedimentation rates during the 1 yr transplantation experiment. Letters above the bars show statistically distinct groups as identified by Tukey's HSD test ($p < 0.05$). AFDW: ash-free dry weight; POM: particulate organic matter

3.2. Ammonia excretion

Significant differences in *L. pertusa* ammonia excretion among the 6 transplantation locations were found (1-way ANOVA: $F(5,30) = 4.38$, $p < 0.01$). However, these differences did not seem to be linked to the distance away from the farm. Ammonia excretion was significantly higher 250 m north of the farm and 2000 m south of the farm compared to fragments collected 750 north of the farm (Tukey's HSD test, $p < 0.01$), while no differences between the other locations were detected. Overall, mean (±SE) ammonia excretion varied among 0.49 ± 0.07 µmol N g AFDW^{-1} h^{-1} 250 m north of the farm to −0.04 ± 0.06 µmol N g AFDW^{-1} h^{-1} 750 m north of the farm (Table 2). As a comparison, ammonia excretion from the baseline measurements in May 2018 was 0.58 ± 0.14 µmol N g DW^{-1} h^{-1} in freshly collected and incubated coral fragments from Kjerringrevet in May 2018, when the transplantation experiment commenced. In our control site (MF), ammonium excretion was 0.65 ± 0.09 µmol N g AFDW^{-1} h^{-1}; at SF, ammonium excretion was −1.36 ± 0.27 µmol N g AFDW^{-1} h^{-1}.

Table 2. Ammonia excretion rate and % destabilized lysosomes of collected coral colonies (mean ± SE, n = 6). Also shown is the growth (budding, mortality and linear growth) of transplanted corals and estimated calcification and net growth (taking into account bioerosion) of *Lophelia pertusa* colonies along a gradient from the fish farm in Julsundet (JS). Different superscript letters show statistically distinct groups as identified by Tukey's HSD test ($p < 0.05$). Weight gain estimated using the conversion factor 30.76. Net growth calculated using the bioerosion rate of fjord reefs from Büscher et al. (2019) and adjusted within the depositional zone of farm waste based on Kutti et al. (2015). DW: dry weight; MF: Midtfjorden; SF: Sunndalsfjorden; na: not analysed

Site	Ammonia excretion (μmol N g DW^{-1} h^{-1})	Destabilized lysosomes (%)	Budding rate (%)	Polyp mortality (%)	Measured	Calculated	
					Linear growth (mm $polyp^{-1}$ yr^{-1})	Weight gain (g m^{-2} yr^{-1})	Net growth (g m^{-2} yr^{-1})
JS A	$0.49 \pm 0.07^{X,Z}$	30 ± 2^{X}	72	4	5.01	154	108
JS B	0.15 ± 0.03^{Z}	17 ± 2^{Y}	66	1	5.23	161	115
JS C	$-0.04 \pm 0.06^{Y,Z}$	$19 \pm 2^{X,Y}$	23	2	3.46	106	60
JS D	0.30 ± 0.19^{Z}	18 ± 3^{Y}	71	2	4.73	145	99
JS E	0.36 ± 0.09^{Z}	17 ± 2^{Y}	135	0	8.40	258	212
JS F	$0.48 \pm 0.06^{X,Z}$	18 ± 4^{Y}	100	1	7.39	227	204
MF	0.65 ± 0.1	20 ± 8	na	na	na	na	na
SF	−1.36 ± 0.3	na	na	na	na	na	na

3.3. Cellular stress

Significant differences in the lysosomal membrane destabilization rates of *L. pertusa* fragments at the 6 transplantation locations were found (1-way ANOVA: $F(5, 24) = 3.63$, $p < 0.05$). Mean % of destabilized lysosomes in *L. pertusa* fragments growing 250 m north of the edge of the fish farm was 30% (Table 2). This was significantly higher than the rates in coral fragments grown 500 and 1000 m north of the farm as well as 1000 and 2000 m south of the farm (17–18%), but not different from the nubbins grown 750 m north of the farm (19%) (Tukey's HSD test, $p < 0.05$). No other differences in LMS among locations were documented. In our transplant control site (MF), 300 m away from the fish farm, the % destabilized lysosomes in *L. pertusa* colonies was 20%. At SF, 500 m away from the fish farm, LMS could not be determined due to a very low number of harvested cells in the analysis.

3.4. Tissue energy reserves

Lipid content in coral fragments varied significantly among locations (1-way ANOVA: $F(5, 24) = 6.08$, $p < 0.01$) (Fig. 3), with a trend of lower lipid concentrations within the depositional footprint of the farm compared to reference stations located outside this area. Lipid concentration was significantly reduced in corals growing 750 m north compared to reference stations 1000 and 2000 m south of the farm (Tukey's HSD test, $p < 0.05$). Mean (±SE) lipid concentration was 0.13 ± 0.01 mg lipid mg tissue DW^{-1} in corals growing within the depositional footprint of the farm, while it was 0.30 ± 0.05 mg lipid mg tissue DW^{-1} in reference corals growing 1000 and 2000 m south of the farm. As a comparison, lipid concentration in the freshly collected coral fragments from Kjerringrevet was 0.27 ± 0.04 mg lipid mg tissue DW^{-1} when the transplantation experiment commenced in May 2018. In our transplant control site (MF), lipid stores in freshly collected *L. pertusa* colonies were 0.26 ± 0.05 mg lipid mg tissue DW^{-1}, while in SF, lipid stores were 0.14 ± 0.02 mg lipid mg tissue DW^{-1} (June 2019).

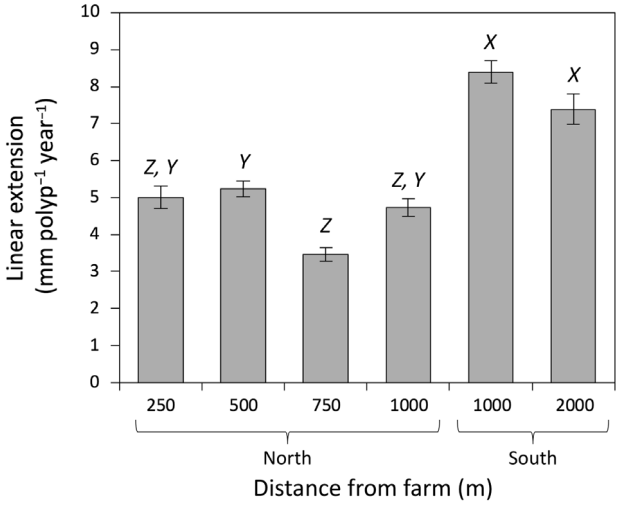

Fig. 3. Lipid content (mean ± SE) of *Lophelia pertusa* coral fragments after 1 yr of transplantation from Kjerringrevet (a nearby cold-water coral reef) to a gradient away from a salmon fish farm. n = 6 for all sites. Letters above the bars show statistically distinct groups as identified by Tukey's HSD test ($p < 0.05$)

3.5. Growth, budding and mortality rates

All *L. pertusa* fragments showed linear skeletal extension, with rates varying significantly among sites (1-way ANOVA: $F(5, 43) = 5.71$, $p < 0.01$) (Fig. 4). Annual linear growth of young polyps was significantly higher for the 2 reference sites (1000 and 2000 m south of the farm) located outside the main plume of particulate waste from the farm than for the rest of the sites (i.e. 250, 500, 750 and 1000 m north) (Tukey's HSD test, $p < 0.001$). Mean annual linear growth at these 2 reference sites was 7.99 mm yr^{-1}, which was 70% higher than mean annual growth at the 4 sites located within the main plume of particulate waste dispersed from the fish farm. Budding rate differed significantly between the reference site 1000 m south and affected sites 500 and 750 m north and the reference site 2000 m south and affected site 750 m north. Highest budding rates were observed at the reference site located 1000 m south of the farm, with 135%, while the lowest budding rate was observed 750 m north of the site, with 23% (Table 2). No difference in polyp mortality among sites was detected (Table 2).

3.6. Correlations between sedimentation of organic waste and coral physiology and biology

Simulations showed a relatively wide spreading of faecal particles from all 3 studied farms (Fig. 1) and POM sedimentation rates at the sites where corals were collected varied between 6.64 and 0.05 g m^{-2} d^{-1} (Table 1). These rates are within those measured at other similar-sized fish farms using sediment traps (Kutti et al. 2007a). A strong negative correlation was observed between the average oxygen consumption of all corals combined (i.e. transplanted *L. pertusa* coral fragments and corals from the 2 natural reefs) and the modelled sedimentation of organic particles dispersed from the fish farms ($t = -2.83$, df = 6, $p < 0.05$, $r = -0.76$). Furthermore, a strong positive correlation was found between the size of lipid reserves and coral oxygen consumption using combined data from the transplanted corals and the natural reefs ($t = 4.72$, df = 6, $p < 0.01$, $r = 0.89$). Strong positive correlations were also documented between growth and oxygen consumption in transplanted corals ($t = 3.58$, df = 4, $p < 0.05$, $r = 0.87$).

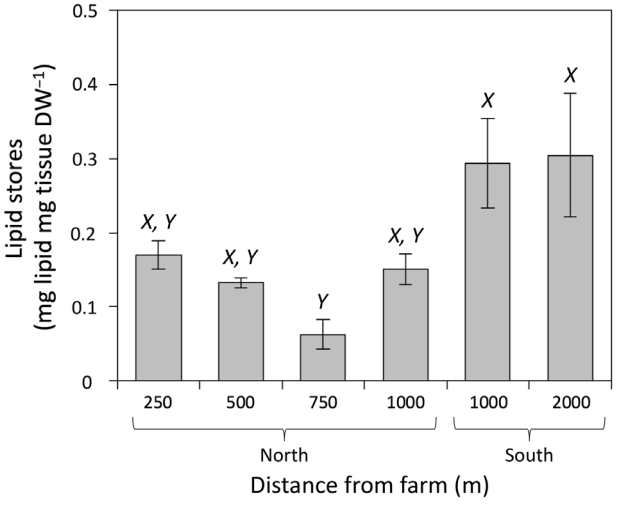

Fig. 4. Linear extension (mean ± SE, mm yr^{-1}) of *Lophelia pertusa* coral fragments after 1 yr of transplantation from Kjerringrevet (a nearby cold-water coral reef) to a gradient away from a salmon fish farm. n = 6 for all sites. Letters above the bars show statistically distinct groups as identified by Tukey's HSD test ($p < 0.05$). DW: dry weight

4. DISCUSSION

4.1. Coral metabolism

Many studies have demonstrated the ability of marine benthic species, including *Lophelia pertusa*, to adjust their metabolic rates to a naturally variable food supply, reducing rates in periods of food limitation and then quickly increasing rates to utilize pulses of food delivered to the sea floor (van Oevelen et al. 2016, Maier et al. 2019, Schuster et al. 2019). The strong negative correlation between the respiration rates of both transplanted and naturally occurring *L. pertusa* and modelled sedimentation rates of organic particles derived from the farms in this study was therefore surprising and demonstrates that despite its opportunistic feeder behaviour (Mueller et al. 2014), *L. pertusa* does not seem to utilize the organic waste released from fish farms as food. Instead, enhanced sedimentation of faecal waste caused up to 60% reduction in mass-specific respiration rates within the depositional footprint of the farms compared to reference stations, the baseline respiration measurement of freshly collected coral fragments from Kjerringrevet CWC reef carried out in May 2018 and other coastal *L. pertusa* reefs (Maier et al. 2020), suggesting metabolic depression. Metabolic depression is a widely observed phenomenon used by organisms to enhance survival in response to environmental stress (reviewed by Guppy & Withers 1999). Reduced respiration rates by up to 50% have been observed in starved *L. pertusa* (Baussant et al. 2017, Maier et al. 2019) and in *L. pertusa* exposed to

elevated pCO_2 (Hennige et al. 2014) in laboratory conditions. Enhanced suspended particle loads close to the farms may have evoked a behavioral response in *L. pertusa* to reduce its polyp extension (as shown by Larsson et al. 2013), which could have caused a subsequent food limitation and reduced respiration rates. The use of *in situ* time-lapse cameras to monitor the coral's polyp behavior (e.g. Osterloff et al. 2019) is warranted in future studies to shed further light on this.

Seasonal changes in metabolic rates driven by an interplay between food availability and bottom water temperature has been documented in *L. pertusa* (Maier et al. 2020) and with this study, demonstrating a consistency between early summer metabolic rates between 2 consecutive years (2018 and 2019). Further, the consistency between metabolic rates, as well as lipid stores, of the freshly collected coral fragments in May 2018 and coral fragments from control stations in the transplantation experiment in June 2019 demonstrate that a time-related degradation of the coral's health condition did not occur as a result of the transplantation. While the analysis of corals naturally growing near fish farms does not provide sufficient information to assess an accumulated impact of consecutive production cycles, it does corroborate the results from the transplantations with higher modelled sedimentation, yielding a stronger metabolic response and larger reductions in energy stores.

In contrast to organic particles, exposure to increased suspended sediment loads of inorganic mineral particles does not seem to depress metabolic rates (Larsson et al. 2013, Baussant et al. 2018), and it has been concluded that *L. pertusa* has a relatively high resilience to enhanced turbidity (Buhl-Mortensen et al. 2015, Purser 2015), a trait likely evolved as a response to natural resuspension events evoked by passing winter storms (van Engeland et al. 2019). Keeping the body surface clean from settling particles is important to prevent anoxic conditions and coenosarc damage and loss. The cleaning process has a low cost for *L. pertusa* and is achieved by the rejection of secreted mucus sheets (Larsson & Purser 2011), affecting neither metabolic rates nor energy stores (Larsson et al. 2013, Baussant et al. 2018). In addition to the active sediment removal, strong currents (as those regularly present at the study sites [Table 1]) will help prevent sediment particles from settling onto the surface of the coral as well as resuspend already-settled particles. Careful ROV video examinations before retrieval revealed that neither transplanted corals nor field controls showed accumulation of sediment or organic particles on their body surfaces nor had farm waste accumulated on the seabed around the corals. It therefore seems unlikely that the metabolic depression observed in this study was caused by factors associated with the degradation of organic material such as anoxia or the release of toxic byproducts released from the farm (Robinson et al. 2016). Coenosarc loss was not observed in any of the retrieved coral fragments upon examination under stereo microscopy.

Nitrogen metabolism of the corals varied substantially in this study and with net uptake of ammonium observed in the natural field population at SF and in corals transplanted to 750 m north of the farm. Fish farm effluents can impact coral physiology and shift the microbial communities associated with the coral holobiont, as observed for the temperate soft coral *Duva florida* (Laroche et al. 2022) and tropical corals (Garren et al. 2009). *L. pertusa* mucus and tissue are associated with a diverse community of microorganisms, with some microorganisms being specific to *L. pertusa*, while other parts of the coral microbiome vary depending on both the habitat and the coral's nutritional state (Neulinger et al. 2008, Kellogg et al. 2009, Chapron et al. 2020). Complex recycling of nitrogen has been demonstrated within the *L. pertusa* holobiont, with ammonium assimilated by microbial symbionts transferred into the coral's tissue (Middelburg et al. 2016). The different ammonia uptake rates in the studied corals indicate spatial differences in the structure of associated microbial communities, and these could be linked to a combination of different local-scale environmental factors (such as local small-scale variability in hydrodynamics), possibly in combination with sedimentation rates of organic farm waste or differences in catabolic substrates used in the coral's metabolism. While the importance of the microbial associates for *L. pertusa* nutrition, metabolism and overall health has not been quantified, one study from the Mediterranean demonstrated that shifts in the coral's microbiome occurring upon translocation do not impact coral growth (Chapron et al. 2020).

Copper originating from salmon feed or the antifoulants of the net cages may accumulate in soft sediments and in flocculent matter settled on hard bottom underneath and near fish farms (Hamoutene et al. 2018, Grefsrud et al. 2021) and consequently impact marine fauna. Copper is essential for all organisms in very small levels (therefore added to the feed) but becomes toxic at higher concentrations. Mandatory environmental monitoring performed in 2017 to 2019 directly beneath the net cages at our studied farms showed copper at concentrations of 7 mg kg^{-1}

sediment at JS, 3 mg kg^{-1} sediment at MF and 30 mg kg^{-1} sediment at SF. Copper concentrations 200 to 600 m away from the farming structures were 3 to 6 mg kg^{-1} sediment at JS, 3 to 6 mg kg^{-1} sediment at MF and 25 to 70 mg kg^{-1} sediment at SF (Hektoen 2017, Kjerstad 2018, Hektoen et al. 2019). Consequently, these studies indicate conditions similar to background values at JS and MF and good environmental status at SF, i.e. with concentrations below that known to cause toxicity in marine fauna (Grefsrud et al. 2021). Copper concentrations in bottom water next to the sea cages are generally well below threshold levels, indicating reference environmental status, i.e. <0.5 to 1.7 µg l^{-1} (B. E. Grøsvik et al. unpubl.). The lethal and sub-lethal tolerance thresholds of *L. pertusa* exposed to suspended copper ions are not known; however, the deep-sea gorgonian coral *Dentomuricea meteor* exhibits lower sensitivity to copper exposure (LC_{50} of 140 µg l^{-1}) in comparison to tropical shallow-water corals (Martins et al. 2018). For example, for *Galaxea fascicularis*, a LC50 of 32 µg l^{-1} has been determined for Cu exposure (Sabdono 2009). Overall, the low levels of cellular stress and low mortality observed around the studied farms indicate that the corals were not exposed to toxic concentrations of copper. Moreover, metabolic depression seems to be an efficient strategy for the corals to enhance their survival in response to the stress of organic loading.

4.2. Energy stores and growth

Metabolic adjustment in *L. pertusa* and other deep-sea organisms has likely evolved as an efficient way of sustaining energy stores during seasonal periods of food limitation (Maier et al. 2019, 2020). When starved for 6 mo in laboratory conditions, *L. pertusa* reduced its metabolic rates (by 50%) while maintaining skeletal growth and energy stores (Baussant et al. 2017). The reduced growth and energy stores observed in this experiment exposing *L. pertusa* to settling waste from the fish farm for 1 yr indicate that metabolic depression may not work as an efficient way of conserving energy stores in the long run. Instead, our findings are consistent with the general opinion that biochemical and/or behavioral changes evoked by depressed metabolic rates will inevitably cause losses of coral biomass (McCue 2010, Jacobson et al. 2016). Furthermore, the timing of suspended particle stress may be of importance. Suspended particles will initiate tentacle retraction, thus preventing corals from feeding efficiently (Larsson et al. 2013). This may be particularly stressful in spring, when *L. pertusa* needs to utilize the settling phytoplankton bloom efficiently for building up its body reserves after spawning in January and February (Brooke & Järnegren 2013), which results in a 50% loss in lipid stores (Maier et al. 2020). *L. pertusa* is known to utilize a range of different prey items successively throughout the year (Duineveld et al. 2004, Mueller et al. 2014, van Oevelen et al. 2016, 2018, Maier et al. 2020). Enhanced polyp activity is initiated during the settling of the spring phytoplankton bloom (Osterloff et al. 2019), and under normal conditions, *L. pertusa* rapidly rebuilds tissue biomass during spring and early summer (Maier et al. 2020) due to its high capacity to assimilate organic carbon (van Oevelen et al. 2016, Maier et al. 2019). By the time the transplanted corals from this study were retrieved, i.e. late June, the total lipid content of reference corals was similar to summertime values reported for other Norwegian *L. pertusa* reefs (Nordbø 2015).

Laboratory experiments have shown that *L. pertusa* growth seems to respond more slowly to changes in food availability compared to tissue energy stores, presumably due to the comparatively low cost of calcification in *L. pertusa* (van Oevelen et al. 2016). Nevertheless, this study shows a strong linear correlation between metabolic rates and growth. Corals that had been exposed to an average sedimentation of 7 g m^{-2} d^{-1} for a full year exhibited on average 70% lower linear growth than corals exposed to sedimentation rates that would be considered normal, i.e. 0.5 to 1 g m^{-2} d^{-1} (Kutti et al. 2007a, Keeley et al. 2019). While the growth reduction observed in corals located within a 1 km radius of the farm in the transplantation experiment (i.e. from 7–8 to 4–5 mm yr^{-1}) may not appear alarming when compared to the range of vertical growth measured on other fjord

Table 3. Linear growth (mean ± SE) from Norwegian coastal and fjord reefs. n: number of polyps that were measured for growth. na: not analysed

Reef	Linear extension (mm $polyp^{-1}$ yr^{-1})	Year	n	Reference
Kjerringrevet	7.01 ± 3.87	2018–2019	233	This study
Nordleksa (on)	1.98 ± 0.57	2013–2014	na	Büscher et al. (2019)
Nordleksa (off)	2.65 ± 1.06	2013–2014	na	Büscher et al. (2019)
Nakken	10.07 ± 6.87	2014–2015	72	Maier et al. (2020)
Røberg	7.03 ± 2.30	1995	3	Mortensen et al. (1998)

reefs, i.e. from 2 to 10 mm yr^{-1} (Table 3), over time, such a growth reduction may have a scaling effect on the CWC reef ecosystem and thus be of high biological and geological significance. The development of CWC reefs is regulated by the combined actions of calcification (growth) of the framework-building coral and biological, physical and chemical processes eroding the coral skeleton, e.g. boring micro-organisms and feeding macrofauna (Beuck et al. 2010, Stevenson & Rocha 2013), sedimentation and extreme hydrodynamic events (Lartaud et al. 2017) and dissolution (Silbiger & Donahue 2015). For an inshore Norwegian reef, a total loss of 23 g *L. pertusa* skeleton m^{-2} yr^{-1} has been measured (Büscher et al. 2019). However, bioerosion rates are known to be dependent on food availability and nutrient levels (see e.g. Carreiro-Silva et al. 2009), and studies have shown that near fish farms (where organic loadings are high), the bioerosion of coral skeleton can be 100% higher than at reference sites (Kutti et al. 2015). Hence, erosion of coral skeleton within the depositional footprint can be as high as 46 g m^{-2} yr^{-1} and the net growth loss 100% when comparing reefs within the depositional footprint of fish farms with reefs outside (Table 2). As reef growth is the result of a complex interplay between coral growth and sedimentation, established and fine-tuned over centuries, disrupted growth may eventually result in burial of the reef structures by sediments.

5. CONCLUSIONS

Combining physiological measurements of corals transplanted onto a gradient away from one salmon farm and of corals from reefs located near other salmon farms, this study provided evidence of a strong and quantifiable negative impact of enhanced organic loading associated with fish farming on *Lophelia pertusa* physiological health. The strong correlation observed between modeled sedimentation rates and the metabolic rate of *L. pertusa* and, furthermore, the strong positive correlation between measured metabolic rates and *L. pertusa* energy stores and growth imply that predictions of the expected short-term impact of aquaculture effluents on *L. pertusa* physiological health and CWC reef development may be possible with further research. Such predictions would help management in making informed decisions for requests to establish new farms near CWC reefs and requests for increasing standing biomass and production rates on existing farms located near CWC reefs. Further field and laboratory studies are warranted to elucidate the relationships between the sedimentation of organic farm waste and coral health status. These studies could for example assess the impact of organic farm waste on coral populations in other environmental settings, the cumulative impact of repeated production cycles and the degree to which the responses observed in this study are caused by organic loading alone or a combination of organic loading, other pollutants released from the farm, such as heavy metals and therapeutants, and small-scale variability in hydrodynamics.

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