Effects and recovery of larvae of the cold-water coral *Lophelia pertusa* (*Desmophyllum pertusum)* exposed to suspended bentonite, barite and drill cuttings

Johanna Järnegren [a,*], Sandra Brooke [b], Henrik Jensen [c]

[a] *Norwegian Institute for Nature Research, P.O. Box 5685 Torgarden, 7485, Trondheim, Norway*
[b] *Florida State University Coastal and Marine Lab, 3618 Costal Highway 98 St, Teresa, FL, 32358, USA*
[c] *Centre for Biodiversity Dynamics, Dept. of Biology, Norwegian University of Science and Technology, N-7491, Trondheim, Norway*

ABSTRACT

Fossil fuel drilling operations create sediment plumes and release waste materials into the ocean. These operations sometimes occur close to sensitive marine ecosystems, such as cold-water corals. While there have been several studies on the effects of energy industry activities on adult corals, there is very little information on potential impacts to their early life history stages. Larval stages of many marine organisms, including cold-water corals use cilia as a means of feeding and swimming, and if these structures become clogged with suspended particulates, the larvae may sink and be lost to the system.

The objective of this study was to understand the response of *Lophelia pertusa* larvae to a different drilling waste components, and assess post-exposure recovery. Larvae of two ages (eight and 21 days) were exposed to a range of concentrations of bentonite, barite and drill cuttings. Larval sensitivity was assessed using the concentration at which 50% of the larvae showed behavioral effects (EC_{50}) or lethal effects (LC_{50}). Larvae showed greatest sensitivity to bentonite, followed by barite and drill cuttings, and also showed age-related responses that differed among the test materials. Post exposure recovery was variable across materials, with larvae exposed to bentonite having the lowest recovery rates. Understanding the vulnerability of early life history stages to human activities can help inform management strategies to preserve reproductive capacity of important marine ecosystems.

1. Introduction

Renewable energy sources and natural gas are the fastest-growing sectors of the energy industry, but petroleum and other liquid fossil fuels still represent the largest source of energy globally, and are expected to remain the dominant fuel sources (>30%) until 2040 and probably beyond (US Energy Information Administration, 2017). Drilling for oil and gas moved into deep waters (>200 m) over half a century ago, and development of ultra deep-water extraction (>1000 m) is likely to continue as continued demand for oil creates economic incentives to expand exploration.

Development of commercial oil extraction involves several stages, which include exploration, ground-truthing geological and acoustic data, production, and possible expansion of a field (Boesch and Rabelais, 1987; Hyne, 2001; Gausland, 2003; Sanzone et al., 2016). This process

requires the drilling of multiple wells, and installation of infrastructure that ranges from large platforms constructed on the seafloor, to floating production units. The latter contain typically two to four drilled wells (Sanzone et al., 2016).

During well construction, 'drilling muds' (DM) are used for cooling, lubricating, maintaining pressure control, and cleaning the drill bit, as well as expelling drill cuttings (DC) from the well. The DM may be water-based, oil-based or synthetic. Water-based are the most commonly used in modern drilling operations as they are the least toxic, but other types are occasionally required. Water-based DM consists of water, a weighting agent (often barite), drilling fluid chemicals and various inorganic salts and organic additives (Caenn et al., 2011).

During the drilling process, the upper layers of material are normally deposited directly onto the seafloor, while the deeper material is pumped up to the rig for processing and/or recycling (Sanzone et al., 2016).

Any DC with residual DM material may be discharged overboard from the rig, transported to shore or injected into a dedicated injection well (Boesch and Rabelais, 1987; Ball et al., 2012), depending on chemical composition, local regulations and ecological sensitivity of the adjacent area. The composition of the materials discharged and current speed and direction will influence their dispersal from the discharge point (Purser and Thomsen, 2012). Further detail on the drilling process can be found in Purser and Thomsen (2012), Cordes et al. (2016) and Sanzone et al. (2016).

On the Norwegian continental shelf, the increase in subsea development, in combination with the ban of discharge of oil-based drilling fluids and the water-based drilling fluids components being permitted (Frost et al., 2006; Neff, 2008; Bakke et al., 2013), has changed the focus from only considering effects on the seafloor to also focus on suspended particulates in the water column.

Drilling operations may co-occur with sensitive marine ecosystems; in the deep sea these include cold-water corals and other slow-growing, long-lived taxa (Cordes et al., 2016). Concern of impacts to cold-water coral reefs from energy industry activities have prompted several environmental field studies, primarily in the north Atlantic (e.g. Lepland and Mortensen, 2008; Purser and Thomsen, 2012; Larsson et al., 2013; Godø et al., 2014; Buhl-Mortensen et al., 2015; Purser, 2015). The dominant reef-building cold-water coral species in the North Atlantic is the stony coral *Lophelia pertusa*, which was recently reclassified as *Desmophyllum pertusum* by Addamo et al. (2016). This is arguably one of the most important cold-water coral species, and has been the focus of extensive research and management efforts. Given the application of this research to non-academic audiences, the more familiar species name *Lophelia pertusa* will continue to be used herein.

Cold-water coral reefs, dominated by *L. pertusa* form extensive reef systems across the North Atlantic, which support high diversity and abundance of associated invertebrates and fishes, some of which are economically important (see Roberts et al., 2009). Laboratory studies showed that adult coral could efficiently remove a range of sediment sizes, even after repeated exposures; however, mortality occurred when polyps were completely smothered or buried (Brooke et al., 2009; Larsson and Purser, 2011). The recommended threshold for coral burial used in many energy industry environmental risk assessment models is 6.3 mm DC (Oil and Gas, 2013). Larsson and Purser (2011) concluded that this level could damage *L. pertusa* colonies when exposed over longer time periods (>3 weeks). To prevent damage to sensitive habitats, the energy industry may transport DC away from *L. pertusa* reefs near Norwegian oil fields using a cutting transport system (Frost et al., 2014; Purser, 2015). Monitoring reefs close to oil fields in Norway indicate that this measure is generally successful in protecting the corals (Purser, 2015).

These mitigation measures, and associated monitoring efforts only take the adult corals into consideration; however, the early life history stages of corals and many other taxa may be very sensitive to natural and anthropogenic suspended sediments (Jones et al., 2015), which can clog feeding and swimming structures. In the North Atlantic, *L. pertusa* spawns between January and March (Brooke and Järnegren, 2013) and the coral larvae are sensitive to elevated sediment loads, including DC (Larsson et al., 2013; Järnegren et al., 2017). Unfavorable conditions during this period could cause loss of the entire annual reproductive output, and eventually undermine reef stability. In Australia, dredging is prohibited during shallow-water coral spawning periods to protect these larval cohorts from elevated suspended sediment. Temporal management has been proposed for *L. pertusa* in Norway (Oil and Gas, 2013; Järnegren et al., 2017) to reduce potential impacts to coral larvae. These approaches are contentious as they suspend expensive operations, with the greatest issues being length of closure period and whether complete cessation is necessary. Keys to these decisions are the duration and sensitivity of coral early life-stages, for which data are sparse or lacking (Jones et al., 2015).

Previous studies on adult scallops have shown effects of suspended cuttings from water-based drilling muds as low as 5 mg l^{-1} in laboratory experiments and 15 mg l^{-1} in the field (Bechmann et al., 2006; Berland et al., 2006), with a proposed no effect concentration (PNEC) of 0.8 mg l^{-1}. PNEC is the concentration below which exposure to a substance is not expected to cause adverse effects in an ecosystem (European Chemicals Bureau, 2003). Smit et al. (2008) developed marine species sensitivity distributions (SSDs) for 15 marine species; they concluded that Hazard Concentration levels for 5% (HC5) for suspended barite (17.9 mg l^{-1}) and bentonite (7.6 mg l^{-1}), the two major components of DM are higher than for DC, and lethal concentrations (LC50) of these materials were 1830 mg l^{-1} and 3010 mg l^{-1}, respectively. None of these studies however, included the larval stages of sessile benthic fauna associated with sensitive habitats such as deep coral reefs.

The objective of this study was to better understand the sensitivities of *L. pertusa* larvae to three common components of particulate drilling wastes. The three test materials display different properties when in suspension with water. Bentonite is used for a number of different purposes, but the type used in drilling operations is milled to a very fine clay which forms a colloid or gel when mixed with water. Some forms of bentonite have non-typical viscosity properties such as thixotrophy, where viscosity decreases with increasing shear, which makes them useful as lubricants. Barite is a mineral that is used by the oil industry as a weighting agent and is heavier and coarser than bentonite. Drill cuttings are a mixture of drilling muds and ground bedrock, so their component parts will vary between operations and locations. Larvae were exposed to a range of concentrations of each material, to define the tolerance limits of *L. pertusa* larvae under normal operational conditions and occurring natural (resuspension from benthic storms) and anthropogenic (accidental release) scenarios. This study also assessed the post-exposure recovery potential of coral larvae, to obtain a more complete understanding of the potential impacts of drilling operations on coral larvae and other zooplankton.

2. Materials and methods

2.1. Materials

In this study, *L. pertusa* larvae were exposed to suspended bentonite, barite, and drill cuttings. Bentonite and barite were obtained from Halliburton, Norway (supplier to the energy industry). Drill cuttings were collected in 2009 from the exploration well 'Trolla' (36" section, drilled with prehydrated bentonite), operated by Det Norske, and stored at −80 °C. To study the effects of the finer size fractions, which potentially spread over longer distances than larger particles, drill cuttings and barite were dry sieved through a stainless-steel mesh to obtain the particle size fraction of ≤63 μm. This size fraction was chosen to ensure compatibility with a previous study on effects of drill cuttings on *L. pertusa* larvae (Järnegren et al., 2017). As bentonite is composed of very fine particles, there was no need for size fractionation.

For the exposure experiments, turbidity was used as a proxy for concentration of suspended materials since the latter would be challenging to measure accurately under the experimental scenarios (small volumes of low concentration). Turbidity was measured as nephelometric turbidity units (NTU), in accordance with international standard methods on turbidity determination (ISO 7027-1, 2016), using a HACH TU5200 turbidity meter. Calibration curves were generated for each material to enable conversion of turbidity readings to concentration (mg l^{-1}). Stock solutions (1000 mg l^{-1}) of each test material were diluted with 1.2 μm filtered seawater to generate a range of concentrations (0, 5, 10, 50, 100 and 500 mg l^{-1}). These data were used to construct a calibration curve that encompassed experimental exposure concentrations. Three replicates were made for each concentration and material, and the turbidity (NTU) of every replicate was measured three times.

2.2. Larval culture

Five colonies of *L. pertusa* were collected from depths of 200–300 m in the Trondheim Fjord using the remotely operated vehicle (ROV) Minerva (Sperre AS) in March 2016. The corals were transferred to a holding tank at the Trondheim Biological Station (NTNU) where they were held in a flow-through tank fed with sand-filtered water pumped directly from the fjord at 100 m depth. They were also fed twice a week with preserved copepods (*Calanus finnmarchicus* from Planktonic). In February 2017 the corals were transferred to a series of five tanks, all of which contained minimum four fragments of each different colony (n = 5). Tanks were held in a cold room at 8 °C, which is the average temperature for coral habitats in the Trondheim Fjord (Brooke and Järnegren, 2013). Spawning occurred on March 7th, after which, fertilized eggs were removed from the tanks by first gently scooping the water into a clean glass jar and then carefully pipetting the embryos into 4 L glass jars with 1.2 μm-filtered seawater. The embryos were never exposed to air and always gently handled. Cultures were composed of embryos from multiple tanks but were all the same age and developmental stage. They were kept at 8 °C at a density of approximately 5 larvae ml^{-1} and cleaned every third day to prevent bacterial development. Larvae were maintained in this manner until used for experiments at eight- and 21-days post-fertilization.

2.3. Experimental design

A stock solution (1000 mg l^{-1}) was created for each material, and diluted with 1.2 μm filtered seawater to create experimental concentrations of 0 (control), 10, 30, 50, 100 and 200 mg l^{-1}.

Glass vials (24 ml) with inert Teflon-sealed caps were used as experimental chambers, with three replicate vials per treatment. To maintain particles in suspension, the vials were attached to a series of paddles that were connected to low speed 12 V motors (Uxcell), and gently rotated at 2 revolutions per minute in a horizontal orientation. Treatment vials were randomly distributed across the paddle systems, each of which could hold up to 12 vials. All experiments were maintained in the cold room at 8 °C and were assessed after 24 h. This time interval was chosen for logistical reasons, but further studies that use realistic exposure-recovery rates would provide additional insight for development of management strategies.

2.4. Monitoring of particle concentration over time

The paddle system was designed to maintain particle concentrations in suspension; however, during initial tests, some suspension reduction was observed through particles adhering to the vial walls. To determine how concentrations of the three different materials changed under experimental conditions, 'blank' (no larvae) experiments were conducted. For each material, three replicate vials of each target concentration (0, 10, 30, 50, 100, 200 mg l^{-1}) was maintained under experimental conditions for 24 h. Turbidity was measured at the start of this time-series experiment, and after 1 h, 3 h, 6 h, 12 h and 24 h. At each time point, the vials were carefully detached from the paddles, placed upright, and 20 ml of the suspension was gently pipetted into a clean vial, without disturbing any flocculant attached to the vial walls. Within 30 min of sampling, the vials were agitated to re-suspend the material and turbidity was measured using a 10 ml sub-sample. Particle concentration was derived using the calibration curves, and percentages of initial (target) concentrations were calculated for each material and each time point.

2.5. Larval exposure experiments

Experimental vials containing treatment concentrations of each material were stocked with 25 larvae. Each treatment concentration and material had three replicates. The eight-day larvae were exposed to bentonite, barite and drill cuttings at starting concentrations of 0, 10, 30, 50, 100 and 200 mg l^{-1}. Due to a shortage of older larvae, a more limited experiment was conducted on the 21-day larvae, using only bentonite and drill cuttings, and fewer experimental starting concentrations (0, 30, 50 and 100 mg l^{-1}). Bentonite was used because interesting results were found in the eight-day exposure, and drill cuttings were used as they are the dominant material released during drilling operations.

After 24 h, the contents of each larval exposure vial were gently transferred to a small counting-chamber and larvae were examined under a Leica DM1000 light microscope. Larvae were placed into one of five categories, ranked from non-affected to progressively worse: Normal larvae (N) were those swimming normally and had no particles attached. These larvae defined "normal speed". Normal with particles (NP) were swimming normally but often with reduced speed compared to N, and had a varying number of particles attached to the larvae or as mucus trails. Abnormal (AN) showed some defect (swimming in circles, misshapen body). Live-clogged (LC) were encased in mucus and/or could not swim but were still moving. Dead larvae (D) were those that showed no cilia movement.

Parallel experiments were performed without larvae to determine how experimental concentrations changed between the start (0 h) and the end (24 h) of the experiment. The additional vials were sub-sampled for turbidity measurements and concentrations of suspended material were derived using the calibration curves.

2.6. Recovery of larvae after exposure

After scoring, all larvae were gently transferred to clean experimental vials filled with 1.2 μm filtered seawater and returned to the cold-room. They were left to recover for 24 h, when they were scored again, using the same methodology and metrics as described previously.

2.7. Statistical modelling

The relationship between the probability of larvae being affected and concentration in the exposure experiments was analysed using generalized linear models (function glm) in the R-package lme4 (Bates et al., 2015). To account for any over-dispersion, logistic regression models were run with a quasi-binomial error and logit link function. The larval data was aggregated within each glass vial (replicate) prior to logistic regression analyses, and the response variable was the number of larvae in a given affected category (or worse) vs. the number of larvae that were of the less affected category (or categories) in each glass vial (replicate). Initially, dose-response curves were modeled for each material and larval age, and a total of 20 statistical models were run. In order to account for this multiple testing a Bonferroni-corrected level of significance was used equal to P = 0.05/20 = 0.0025 (Rice, 1989). Next, to examine whether the dose-response curves differed with larval age, results from experiments at both ages (i.e. eight- and 21-day larvae) were included in the same model; one model for bentonite and one for drill cuttings. These models were logistic regressions as described above, but were run for only affected categories "NP or worse", and "LC or worse" (i.e. four models with interactions were run, and we did not adjust the level of significance due to multiple testing in this case). The models included as explanatory variables an interaction term between concentration and age, in addition to the main effects of concentration and age. An interaction term shows that the slopes of the curves differ. Furthermore, the approximate concentration at which there was no longer an overlap between the 95% confidence intervals for the dose-response curves of eight- and 21-days old larvae was used as a conservative measure for when the effect was different for eight-days old and 21-days old larvae. The assumptions of generalized linear models we ran were evaluated by visual inspection of diagnostic plots: residuals versus fitted values (to check for linearity between transformed expectation of response and predictor), QQ-plot (to check for normality of the

response distribution given the model), spread-location plot (to check the assumption of equal variance), and residuals versus leverage plot (to check whether any vials were outliers).

3. Results

3.1. Calibration curves

The calibration curves generated for each material (Fig. 1) show a clear linear relationship between concentration and measured turbidity. The following regression equations were generated for each material, where y = turbidity (NTU) and x = particle concentration (mg l^{-1}).

Bentonite: y = 0.2208x – 0.1575
Barite: y = 0.404x – 0.7756
Drill cuttings: y = 0.2644x + 0.6828

3.2. Changes in experimental concentrations over time

Time-series measurements from the 'blank' experiment show that all concentrations of all materials declined under experimental conditions. Bentonite (Fig. 2A), dropped to an average of 69.89% (SD = 11.73) of the initial (target) concentration during the first hour. After 24 h, the average final concentrations of bentonite were 51.45% (SD = 9.67) of the initial concentration. Barite (Fig. 2B) concentrations declined to an average of 51.63% (SD = 13.15) of the initial concentration after 1 h, and an average of 47.69% (SD = 3.48) after 24 h. Drill cuttings (Fig. 2C) displayed less initial reduction in concentration than the other materials, with an average of 78.96% (SD = 10.66) of the initial concentration remaining after 1 h and an average of 53.54% (SD = 9.31) after 24 h.

Measurements taken at the start and end of each experiment show similar percentage changes as the time series experiment. After 24 h, the average concentrations of bentonite were 47.65% (SD = 8.14) of the starting concentrations for the 8-d larval experiment (Fig. 3A), and 54.32% (SD = 9.37) for the 21-d larvae (Fig. 3D). For barite, the average concentration after 24 h was 54.03% (SD = 8.26) of the target concentration for the 8-d larval experiment (Fig. 3B). The drill cutting experiment showed a final average of 31.53% (SD = 5.39) of the target concentration for the 8-d larval experiment (Figs. 3C) and 52.28% (SD = 9.53) for the 21-d experiment (Fig. 3E).

3.3. Larval response to exposure

Since the actual treatment concentrations (post exposure period) differed substantially from the target concentrations, data presentation and statistical analysis will use the actual concentrations, not the target treatment levels. To avoid the confusion of presenting treatment-specific

concentrations for each material, treatment levels will be used as follows: Control, Level 1, Level 2, Level 3, Level 4 and Level 5, which correspond to the target concentrations 0, 10, 30, 50, 100 and 200 mg l^{-1}. The actual concentrations for each treatment level are presented in Table 1.

3.3.1. Experiment with eight-day larvae

Table 1 shows the target and actual concentrations for all experiments and treatment levels. Table 2 summarizes the average (and standard deviation) percentage of each larval response category, for each treatment level tested for the three target drilling waste materials. Fig. 4 shows a graphical representation of these data.

For the bentonite experiment with the eight-day larvae, the control had 100% normal larvae (N) (Fig. 4A). For the lowest exposure treatment (7 mg l^{-1}), 79.3% (SD = 18.3) of the larvae had particles attached (NP) but were swimming normally (Fig. 4B). At 18 mg l^{-1} (Fig. 4C), the percentage of normal larvae with particles was similar to the lower concentration (75.5%; SD = 9.9), but the larvae were swimming slower. The percentage of NP category was similar (45.1–57.6%) at higher exposure concentrations (26–75 mg l^{-1}), but there was a progressive decrease in swimming speed observed with increasing concentration (Fig. 4D–F). At 54 mg l^{-1} and 75 mg l^{-1}, there was very little cilia movement observed. These non-swimming larvae were scored NP as they still had ciliary movement and were not clogged with particles (LC). In the 75 mg l^{-1} concentration, 35.4% (SD = 25.3) of the larvae were alive (cilia were moving) but clogged (LC) with bentonite and mucous, which formed a capsule around the larva and appeared to prevent them swimming. A small percentage (3.5; SD = 3.1) of the larvae were dead. The bentonite particles appeared attached primarily to the cilia rather than the body of the larva.

In the barite experiment, both the control (Fig. 4A) and the 6 mg l^{-1} (Fig. 4B) showed high percentage (>97%, Table 1) of normal larvae. The 16 mg l^{-1} treatment (Fig. 4C) showed 26.5% (SD = 14.3) of larvae were categorized as NP. The particles did not appear to hinder or slow larval swimming until the experimental concentration reached 55 mg l^{-1}, when 95.0% (SD = 5.0) of the larvae had particles attached (Fig. 4E). At 88 mg l^{-1}, 92.0% (SD = 4.5) of larvae were categorized as NP (Fig. 4F), but larvae were swimming very slowly and had mucus-strings attached. A low percentage (5.7%; SD = 0.8) of larvae were clogged with particles (LC) but none were dead (D). Barite particles appeared to attach more to the body of the larvae than the cilia and larvae did not create mucus capsules, as they did with bentonite.

The drill cuttings experiment had a high percentage of normal larvae in the control (94.5%, SD = 0.7) (Fig. 4A). A small percentage of normal larvae with particles were observed in the 6 mg l^{-1} (2.8%; SD = 4.8) (Figs. 4B) and 12 mg l^{-1} (6.7%; SD = 7.9) (Fig. 4C) treatments. The number of affected (NP) larvae increased to 22.5% (SD = 22.0) in the 18 mg l^{-1} treatment (Fig. 4D), and the larval swimming speed was slower
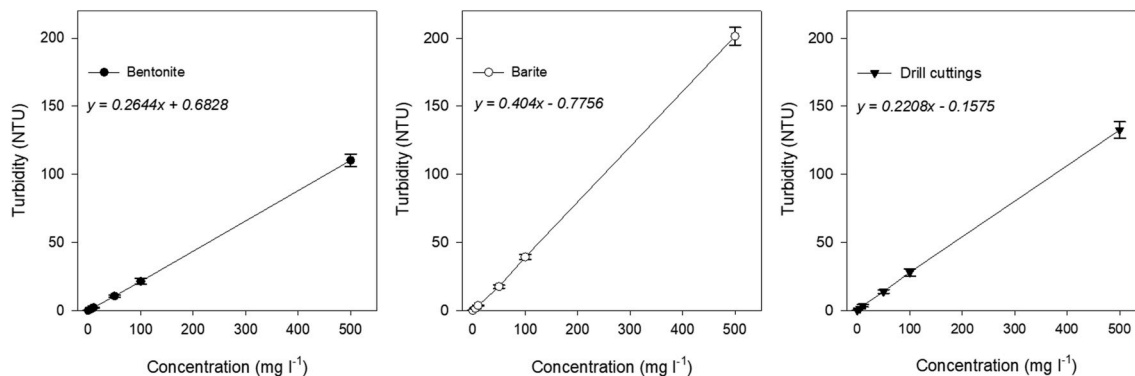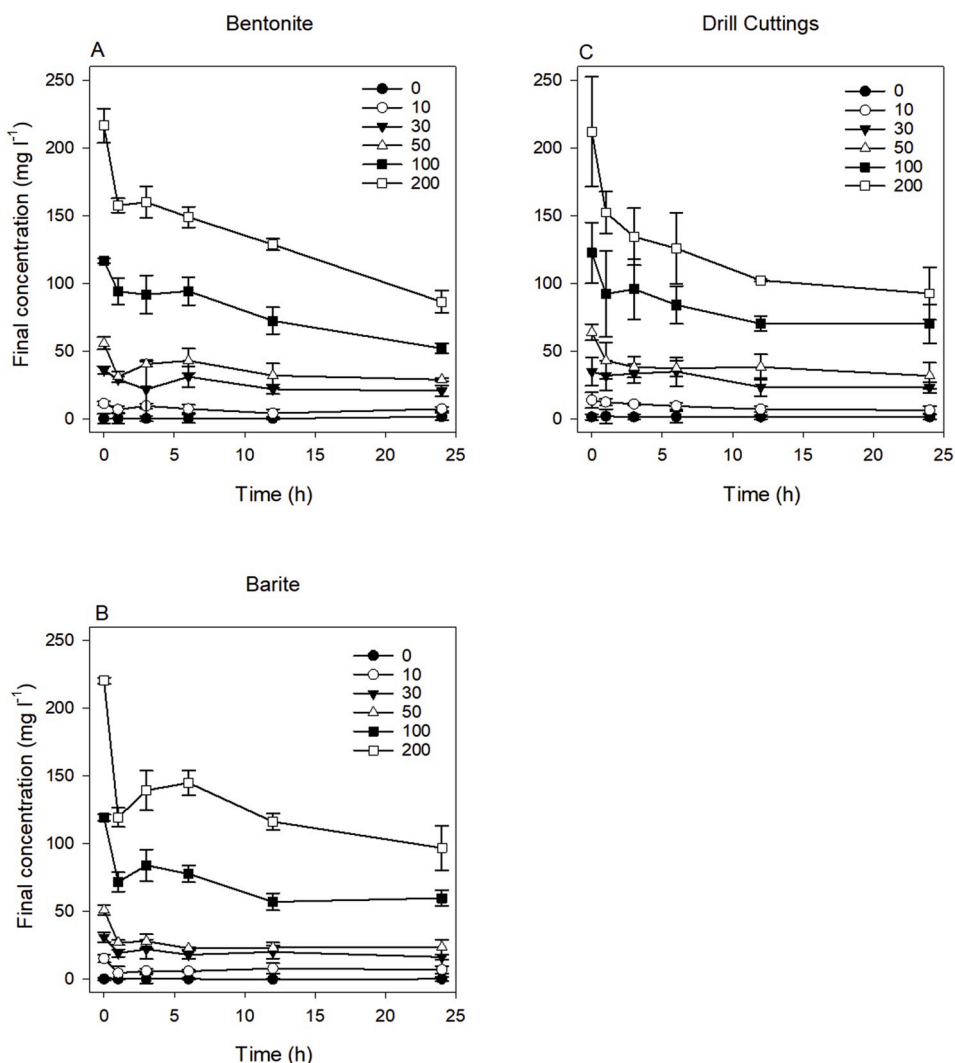

Fig. 1. Calibration curves for bentonite (top), barite (middle) and drill cuttings (bottom), using known concentrations of each material (X-axis) and corresponding turbidity values (Y-axis). Regression equation for each material shown.

Fig. 2. Temporal changes in concentrations of three test materials under experimental conditions: A) bentonite, B) barite, C) drill cuttings. Concentrations were calculated from turbidity measurements taken at time intervals of 1, 3, 6, 12 and 24 h after the start of the experiment. Initial experimental concentrations were 0 (control), 10 mg l^{-1}, 30 mg l^{-1}, 50 mg l^{-1}, 100 mg l^{-1}, 200 mg l^{-1}.

than control larvae. At 32 mg l^{-1}, 27.7% (SD = 6.8) of larvae were classified as NP. At the highest concentration (62 mg l^{-1}), the percentage of larvae with particles increased to 70.4% (SD = 16.0) (Fig. 4F), all the larvae were swimming slowly, and some had mucus strings attached. The percentage of live clogged larvae in the 62 mg l^{-1} treatment was low (3.7; SD = 6.4), and no dead larvae were observed.

3.3.2. Experiment with 21-d larvae

Table 1 shows the target and actual concentrations for the experiments and treatment levels. Table 2 summarizes the average (and standard deviation) percentage of each larval response category, for each treatment level tested for the three target materials. Fig. 5 is a graphical representation of these data.

In the bentonite experiment, the control had 97% (SD = 2.6) normal larvae (Fig. 5A). In the 21 mg l^{-1} (Figs. 5B) and 29 mg l^{-1} (Fig. 6C) treatments, 100% (SD = 0.0) of the larvae had particles attached (NP), occasionally with mucus strings dragging behind. In the 52 mg l^{-1} treatment (Figs. 5D), 55.9% (SD = 13.2) of the larvae were classified as NP as they were swimming normally but very slowly. In this treatment, 17.8% (SD = 13.6) were classified as LC and were trapped in a bentonite-mucus capsule, while 26.2% (SD = 20.0) were dead.

For the drill cutting experiment, the control had 95.4% (SD = 4.5) normal larvae (Fig. 5A). The 23 mg l^{-1} treatment had 22.4% (SD = 10.7)

of larvae with particles attached (NP) and 1.9% (SD = 3.2) were dead (Fig. 5B). In the 32 mgl^{-1} treatment, 30.9% (SD = 30.3) of larvae had particles attached (NP) and a low levels of abnormal (AN) and dead (D) larvae (Fig. 5C). At the highest concentration (70 mg l^{-1}), 78.6% (SD = 8.9) were classified as NP and 2.0% (SD = 3.4) were dead (Fig. 5D). No live clogged larvae were found in this experiment.

3.4. Larval recovery

3.4.1. Recovery of eight-day larvae

Table 3 summarizes the average (and standard deviation) percentage of each larval response category, for each treatment level tested for the three target materials, after 24 h recovery in clean seawater. Fig. 6 is a graphical representation of these data.

After recovery in the bentonite experiment the control had 98.2% (SD = 3.0) normal larvae and 1.8% (SD = 3.0) abnormal larvae. In the 7 mg l^{-1} treatment, 89.8% (SD = 9.3) were normal while 4.5% (SD = 7.9) still had particles attached and 5.7% (SD = 6.3) were abnormal. At the highest concentration (75 mg l^{-1}), 22.7% (SD = 21.1) of larvae were normal while 19.4% (SD = 33.7) still had particles attached, 24.2% (SD = 21.0) were abnormal and 33.7% (SD = 32.3) were live clogged. All larvae except the live clogged were swimming at normal speed in all concentrations.
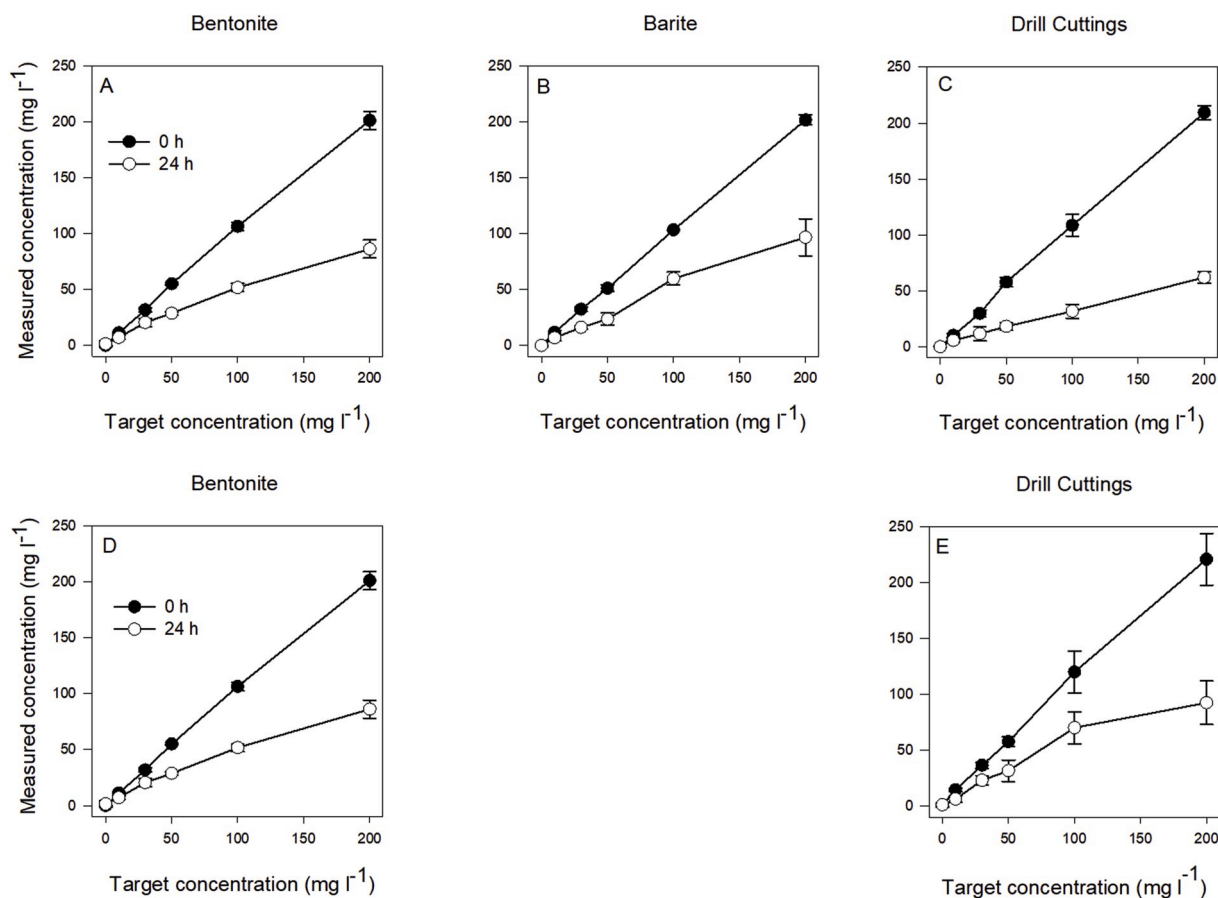
Fig. 3. Measured vs target concentrations at the start (0 h) and end (24 h) of the exposure experiment with eight-day larvae A) bentonite, B) barite and C) drill cuttings, and 21-day larvae E) bentonite and B) drill cuttings.

Table 1
Measured end concentrations after 24 h and target concentration for each material, expressed as level Control and 1 to 5 for eight-day larvae and 21-day larvae. SD = Standard deviation.

Level	Bentonite				Barite		Drill cuttings			
	8-day		21-day		8-day		8-day		21-day	
	(mg l-1)	SD	(mg l-1)	SD	(mg l-1)	SD	(mg l-1)	SD	(mg l-1)	SD
Control	0.0	0.3	0.0	2.8	0.0	0.6	0.0	0.3	0.0	1.9
1	7.0	0.2			6.0	0.5	6.0	1.6		
2	18.0	4.2	20.5	3.9	16.0	2.8	12.0	6.3	23.1	4.0
3	26.0	1.9	28.8	1.4	27.0	1.6	18.0	3.3	31.7	9.5
4	54.0	16.9	51.8	3.5	55.0	9.5	32.0	6.3	70.0	14.3
5	75.0	5.7			88.0	7.2	62.0	5.1		

After recovering from the barite exposure, $\geq 82.0\%$ of larvae were normal across all concentrations, except for 27 mg l^{-1}. At this concentration 57.6% (SD = 16.8) were normal, 47.3% (SD = 10.8) had particles attached and 3.7% (SD = 6.4) were abnormal. There were no live clogged or dead larvae found and all larvae were swimming at normal speed.

After recovery from the drill cuttings exposure, $\geq 86.7\%$ of the recovered larvae were normal at all concentrations. There were no live clogged or dead larvae found and all larvae were swimming at normal speed.

3.4.2. Recovery of 21-day larvae

Table 3 summarizes the average (and standard deviation) percentage of each larval response category, for each treatment level tested for the three target materials, after 24 h recovery. Fig. 7 is a graphical representation of these data.

After recovery from bentonite exposure, $\geq 93.7\%$ of larvae were normal in concentrations up to 29 mg l^{-1}. At 52 mg l^{-1}, 74.8% (SD = 13.5) of larvae were normal, 12.4% (SD = 6.8) had particles attached and 12.9% (SD = 14.5) were live clogged. Only the 21 mg l^{-1} treatment showed mortality, with 1.5% (SD = 2.6) dead. Most larvae were swimming at normal speed, except the live clogged larvae, which were encapsulated in particles.

After recovery from exposure to drill cuttings, $\geq 92.7\%$ of larvae were normal in all four concentrations. However, 2.4% (SD = 4.1) of larvae in the 70 mg l^{-1} treatment had particles attached. No live clogged or dead larvae were found in any concentration. All larvae were swimming at normal speed.

3.5. Statistical modelling

The logistic regression analyses showed that the probability of larvae

Table 2
Mean percentage of larvae of each age in each 'effect' category for all materials and treatments. N - Normal, NP - Normal with particles, AN - Abnormal, LC - Live clogged, D - Dead. SD - Standard deviation.

Level	Effect	Bentonite				Barite		Drill cuttings			
		8-day		21-day		8-day		8-day		21-day	
		%	SD	%	SD	%	SD	%	SD	%	SD
Control	N	100.0	0.0	97.0	2.6	98.4	2.7	94.5	0.7	95.4	4.5
	NP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	AN	0.0	0.0	3.0	2.6	1.6	2.7	5.5	0.7	4.6	4.5
	LC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	N	14.7	15.2			97.1	2.5	89.5	5.8		
	NP	79.3	18.3			0.0	0.0	2.8	4.8		
	AN	4.5	7.9			2.9	2.5	7.7	2.2		
	LC	0.0	0.0			0.0	0.0	0.0	0.0		
	D	1.4	2.5			0.0	0.0	0.0	0.0		
2	N	23.0	12.4	0.0	0.0	73.5	14.3	84.5	7.1	70.3	7.5
	NP	75.5	9.9	100.0	0.0	26.5	14.3	6.7	7.9	22.4	10.7
	AN	1.4	2.5	0.0	0.0	0.0	0.0	8.8	1.0	5.5	5.6
	LC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.9	3.2
3	N	42.4	13.3	0.0	0.0	12.8	6.1	74.0	24.2	55.5	28.3
	NP	57.6	13.3	100.0	0.0	87.2	6.1	22.5	22.0	30.9	30.3
	AN	0.0	0.0	0.0	0.0	0.0	0.0	3.4	3.0	6.3	5.9
	LC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.3	6.3
4	N	44.3	12.9	0.0	0.0	0.0	0.0	57.2	8.6	15.9	4.7
	NP	45.1	33.1	55.9	13.2	95.0	4.7	27.7	16.0	78.6	8.9
	AN	0.0	0.0	0.0	0.0	3.3	2.9	12.7	7.7	3.5	6.1
	LC	4.3	7.5	17.8	13.6	0.0	0.0	0.0	0.0	0.0	0.0
	D	6.2	5.4	26.2	20.2	1.7	2.9	2.4	4.1	2.0	3.4
5	N	5.8	5.6			0.0	0.0	16.7	5.6		
	NP	51.6	33.1			92.0	4.7	70.4	16.0		
	AN	3.7	6.4			2.2	3.8	9.3	8.5		
	LC	35.4	25.3			5.7	0.8	3.7	6.4		
	D	3.5	3.1			0.0	0.0	0.0	0.0		

being affected (i.e. any classification other than N) increased with concentration for all three substances, at both eight- and 21-days of age, except for eight-day larvae exposed to bentonite (Fig. 8; Supplementary Material, Table S1).

The probability of eight-day larvae being severely affected (classified as live clogged (LC) or dead (D)) by exposure, increased significantly with concentration for larvae exposed to bentonite and barite, but not drill cuttings (Fig. 8; Table S1). Similarly, the probability of 21-day larvae being affected (i.e. in category LC or D) increased significantly with concentration for larvae exposed to bentonite, but not drill cuttings (Fig. 8; Table S1). The 21-day larvae were not exposed to barite in the present study.

In the bentonite treatments, there was a significant interaction between age and concentration for the probability of larvae being affected (i.e. any classification other than normal; interaction: p = 0.031), these results suggest that bentonite has a more severe effect on 21-day larvae than on eight-day larvae (Fig. 8A). Moreover, there was a tendency that the probability of larvae being severely affected also differed between ages (i.e. classified as live clogged or dead; interaction: p = 0.096) when exposed to bentonite (Fig. 8D). Based on when their 95% confidence intervals no longer overlap, the effect was similar for the two ages up to approximately 45 mg l^{-1}, after which the older larvae became significantly more affected than the younger larvae (Fig. 8D, Table 2).

In contrast, there was no significant interaction between age and concentration for the probability of larvae being affected (i.e. any classification other than normal) in the drill cuttings treatments (Fig. 8C; interaction: p = 0.598). There was therefore no difference in the effect of drill cuttings on eight-day versus 21-day larvae. Similarly, the probability of larvae being severely affected (classified as LC or D) when exposed to drill cuttings, showed no significant difference between the two ages (Fig. 8F; interaction: p = 0.173).

3.5.1. Derivation of EC10/20/50 values

The concentration level at which 10, 20 and 50% of the larvae were affected (any classification other than N) is referred to as the Effect Concentration (EC10, EC20, EC50). These concentrations were calculated from the logistic regression models using target concentrations (Table S1). The EC10, 20 and 50 values for each material and larval age are presented in Table 4.

According to the model, for eight-day larvae exposed to bentonite, the EC10 was 0 mg l^{-1}, the EC20 was 0 mg l^{-1} and EC50 was 10 mg l^{-1}. For the 21-day larvae, the bentonite EC10 was 3 mg l^{-1}, the EC20 was 6 mg l^{-1} and the EC50 was 10 mg l^{-1}. For eight-day larvae exposed to barite, the EC10 was 11 mg l^{-1}, the EC20 was 14 mg l^{-1} and the EC50 was 20 mg l^{-1}. For eight-day larvae exposed to drill cuttings, the EC10 was 5 mg l^{-1}, the EC20 was 16 mg l^{-1} and the EC50 was 37 mg l^{-1}. For 21-day larvae, the EC10 was 4 mg l^{-1}, the EC20 was 17 mg l^{-1} and the EC50 was 40 mg l^{-1}.

3.5.2. Derivation of LC10/20/50 values

The concentration level at which 10, 20 and 50% of the larvae are dead (LC or D) is referred to as the Lethal Concentration (LC) for each level (LC10, LC20, LC50). These concentrations were calculated from the logistic regression models using target treatment concentrations (Table S1). The LC10, 20 and 50 for each material and larval age are presented in Table 4.
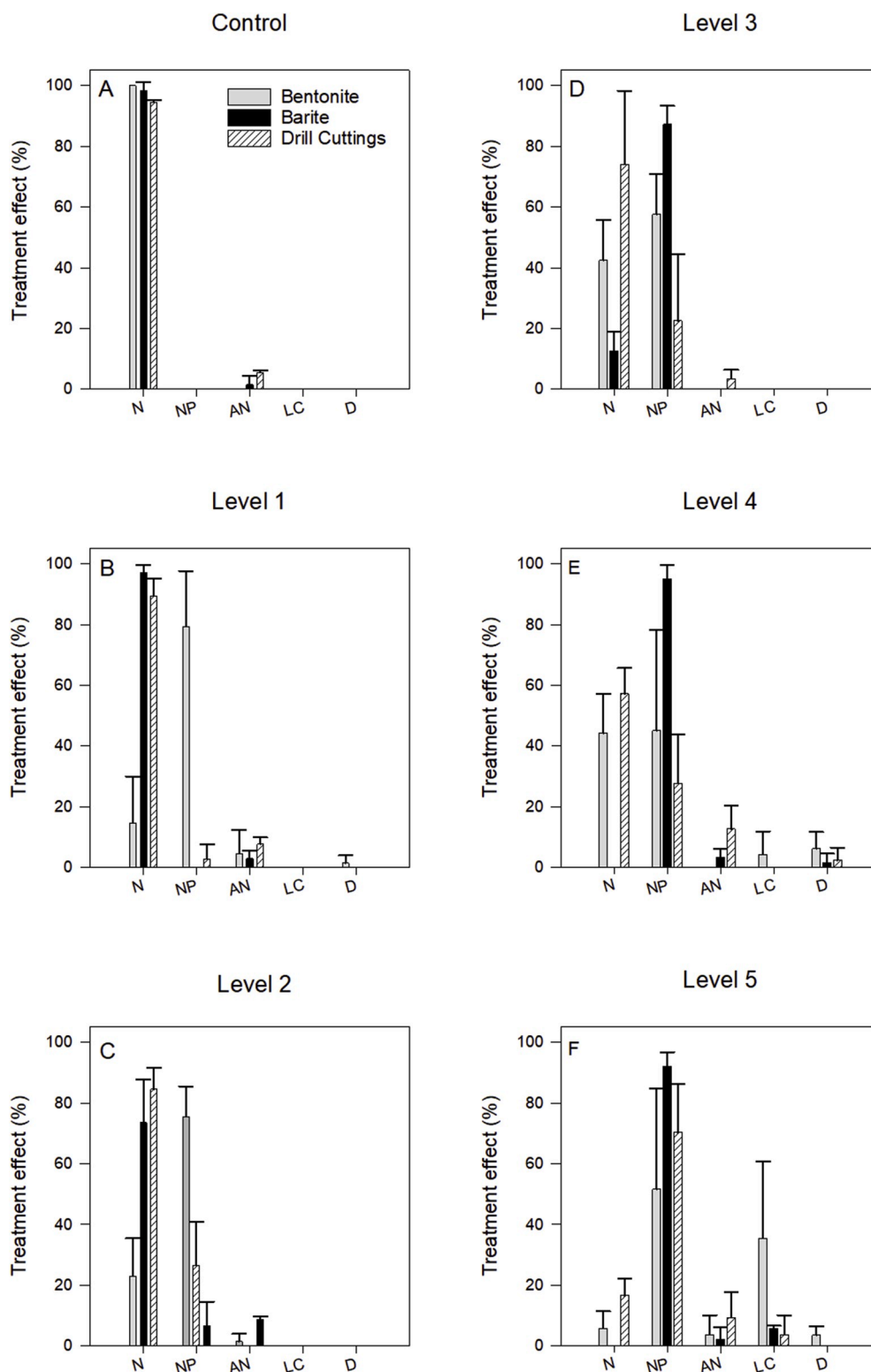
Fig. 4. Treatment effects (% of each response category) on eight-day larvae after exposure to a range of concentrations of three test materials: bentonite, barite and drill cuttings. Each panel represents a single exposure concentration. Response categories: N = normal, NP = normal with particles, AN = abnormal, LC = live clogged, D = dead. Level values for each material are found in Table 1.

According to the model, for eight-day larvae exposed to bentonite, the LC10 was 53 mg l^{-1}, the LC20 was 63 mg l^{-1} and LC50 was projected to be 80 mg l^{-1}. For the 21-day larvae, the bentonite LC10 was 41 mg l^{-1}, the LC20 was 45 mg l^{-1} and the LC50 was 53 mg l^{-1}. For eight-day larvae exposed to barite, the LC values were all higher than the maximum treatment concentration, and were therefore derived from model projections. The LC10 was 97 mg l^{-1}, the LC20 was 110 mg l^{-1} and the LC50 was 133 mg l^{-1}. As for the barite, the LC values for eight-day larvae exposed to drill cuttings exceeded the highest treatment concentration. The LC10 was 77 mg l^{-1}, the LC20 was 90 mg l^{-1} and the
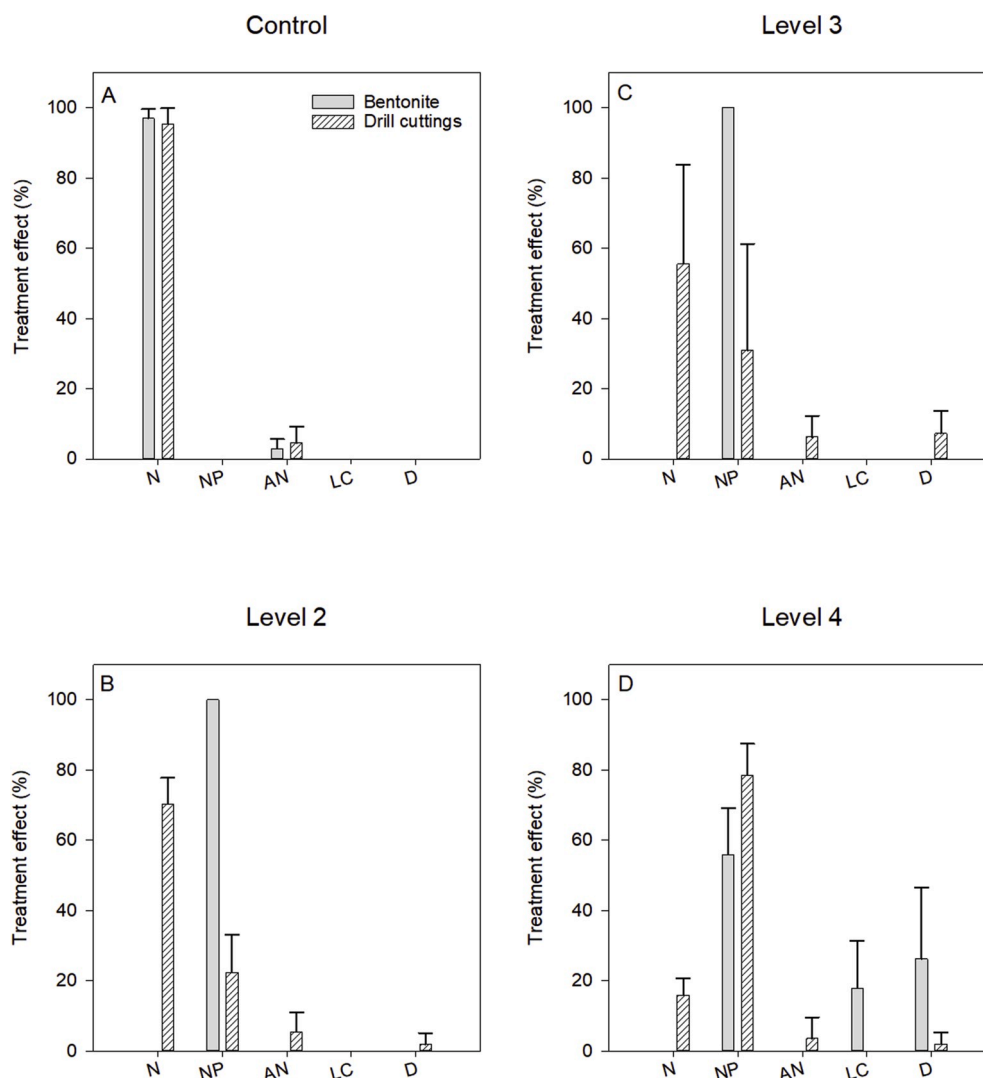
Fig. 5. Treatment effects (% of each response category) on 21-day larvae after exposure to a range of concentrations of two test materials: bentonite and drill cuttings. Each panel represents a single exposure concentration. Response categories. N = normal, NP = normal with particles, AN = abnormal, LC = live clogged, D = dead. Level values for each material are found in Table 1.

LC50 was 112 mg l^{-1}. For the 21-day larvae, the LC10 was 170 mg l^{-1}, the LC20 was 248 mg l^{-1} and the LC50 was 380 mg l^{-1}.

4. Discussion

The calibration curves show that each material has a different concentration-turbidity relationship, with barite having the highest turbidity value for a given concentration, followed by drill cuttings and bentonite (the latter two being very similar). Under experimental conditions, the test concentration of barite declined quicker than the other two materials, which can be explained by its higher density; however, the bentonite concentration declined slightly more quickly than drill cuttings, which was unexpected as the drill cuttings were coarser than the bentonite and therefore were expected to settle out of the suspension more quickly. In the experimental situation, the constant rotation of the glass vials prevented settlement, and the 'lost' material appeared to adhere to the walls of the glass. It is possible therefore, that bentonite has the tendency to stick to the glass surface more quickly than drill cuttings, explaining the faster drop in concentration. At the end of the 24-h time series experiment, however, the concentrations of all materials were approximately half of their original levels. In a natural field situation, the rate of material settlement will depend on factors such as current speed and particle density, but for the sake of discussion, the observed experimental settlement rates were assumed to be similar to a field situation.

The reduction in material concentration during the experiment adds a level of uncertainty to the results. The observed effects may have occurred immediately and persisted until the end of the experiment; alternatively, initial exposure responses to the target concentrations may have been modified over the experimental exposure period because of the changing concentrations. These hypotheses could be tested by conducting a series of experiments with increasing time periods, but this is logistically challenging and requires a large quantity of larvae. In the absence of other information, the more conservative outcomes should be used for management purposes.

Model outputs for bentonite shows an EC50 for both eight-day and 21-day larvae at concentrations ~10 mg l^{-1} (Table 4). All of the control larvae in the eight-day experiment were normal, but high numbers of affected larvae were observed in Level 1 and 2 treatments, with lower numbers in Levels 3 and 4 (Table 2). This pattern of effects flattened the model curve for the eight-day larvae and forced a non-zero origin (Fig. 8A). Despite this statistical artefact, the experimental data (Table 2) supports the model outputs, which indicate that bentonite has negative impacts on eight-day larvae at low concentrations, and that 21-
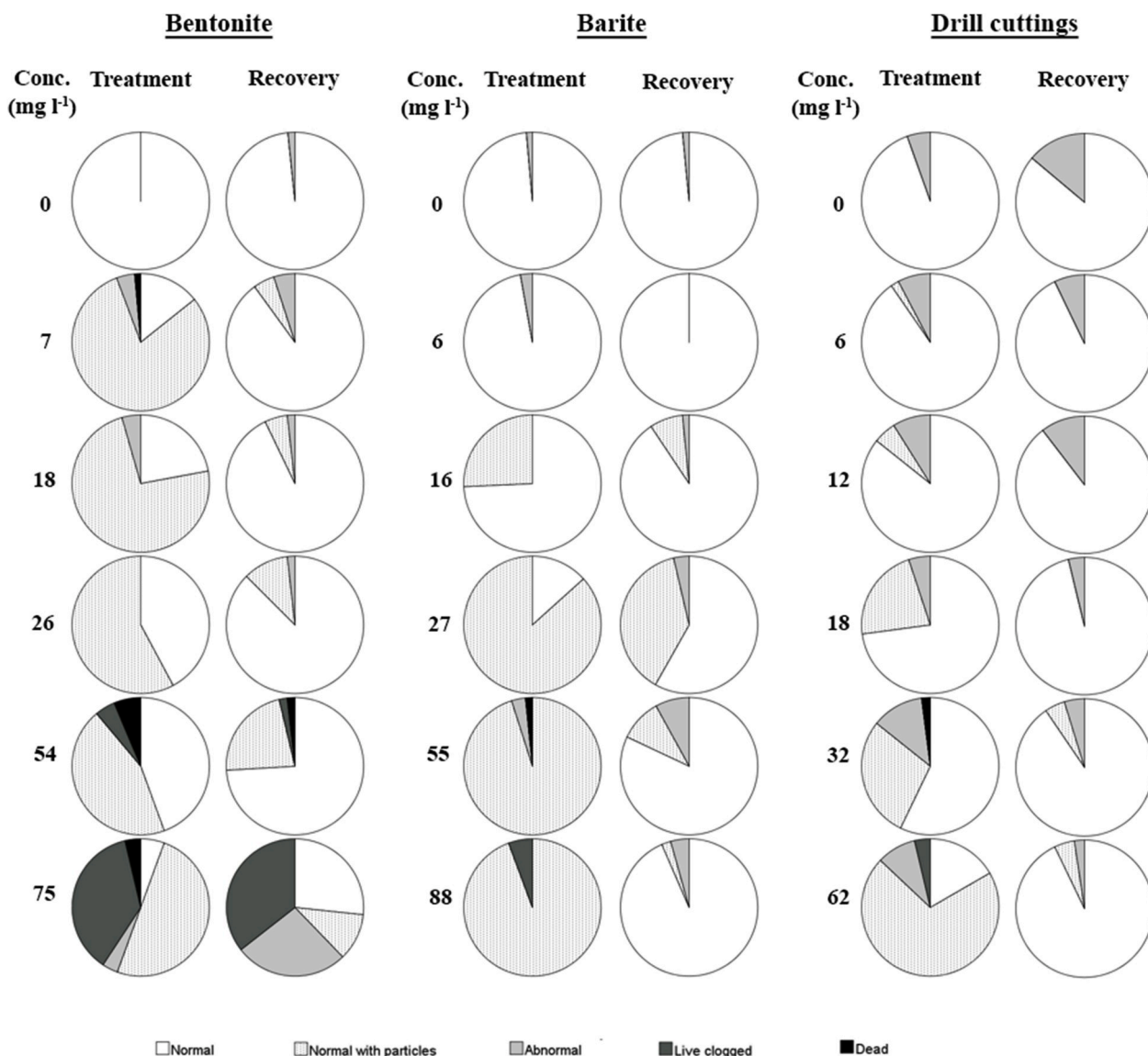
Fig. 6. Treatment and recovery effects (% of each response category) of three test materials on eight-day larvae. Both treatment and recovery period lasted 24 h. Each pair diagram represents response after exposure and recovery from a single concentration. Response categories: N = normal, NP = normal with particles, AN = abnormal, LC = live clogged, D = dead.

larvae are even more affected than the younger larvae (Fig. 8A). The 'severe effects' model (Fig. 8D) showed a higher probability of impacts to 21-day larvae (LC50 = 53 mg l^{-1}) than eight-day larvae (LC50 = 79.5 mg l^{-1}). The experiments therefore showed clear age-related differences in response to bentonite suspensions.

The EC50 for barite was 20 mg l^{-1} for eight-day larvae, which is 2 times higher than for bentonite. The 'severe effects' (LC) values were derived from the statistical model, which generated an LC50 value of 133 mg l^{-1}. This is beyond the highest experimental concentration and is therefore a predicted value based on the statistical model of the experimental data. An assessment of the environmental risk of drilling discharges (Smit et al., 2008), used published data on sensitivities of marine species to different drilling components, to develop marine species sensitivity distributions (SSDs) based on acute effects data. The hazardous concentrations (HC50) for suspended barite and bentonite were 3010 mg l^{-1} and 1830 mg l^{-1}, respectively. The (equivalent) LC50 from our study for barite was 133 mg l^{-1} (eight-day larvae) and 80 mg l^{-1} (eight-day larvae) and 53 mg l^{-1} (21-day larvae) for bentonite. Larvae in this study were 23 times more sensitive than the Smit et al. (2008) values for barite and 23–35 times higher for bentonite. This

disparity highlights the need for more research on larvae and other plankton to obtain a more comprehensive understanding of the effects of contaminants in marine ecosystems.

The experimental data and statistical model outputs for drill cuttings showed the highest EC and LC values of the three test materials (Table 4, Fig. 8C). The EC values indicated that both ages have similar sensitivity to suspended drill cuttings. The LC50 values however, show that eight-day larvae are more sensitive than 21-day larvae. A previous study on the effects of drill cuttings on *L. pertusa* larvae (Järnegren et al., 2017) reported that the dose at which 50% of larvae were 'affected' was 330 mg l^{-1} for five day larvae and 280 mg l^{-1} for 15-day larvae, indicating a higher sensitivity in older larvae. This earlier study defined 'affected' as live clogged or dead, which is the equivalent of a severe effect in the current study. The earlier study had two objectives, the first was to develop a method that would maintain material in suspension without damaging delicate coral larvae, and the second was to define the tolerances of *L. pertusa* larvae to suspended drill cuttings. This study was expanded to consider the effects of additional materials (bentonite and barite), and to account for possible loss of suspended material during the exposure period, thereby creating more accurate experimental results.

Table 3
Mean percentage of larvae of each age in each 'effect' category after recovery for 24 h for all materials and treatments. N - Normal, NP - Normal with particles, AN - Abnormal, LC - Live clogged, D - Dead. SD - Standard deviation.

Level	Effect	Bentonite				Barite		Drill cuttings			
		8-day		21-day		8-day		8-day		21-day	
		%	SD	%	SD	%	SD	%	SD	%	SD
Control	N	98.2	3.0	96.4	3.2	98.2	3.0	86.7	17.6	96.2	3.4
	NP	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	AN	1.8	3.0	3.6	3.2	1.8	3.0	13.3	17.6	3.8	3.4
	LC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	N	89.8	9.3			100.0	0.0	92.6	1.6		
	NP	4.5	7.9			0.0	0.0	0.0	0.0		
	AN	5.7	6.3			0.0	0.0	7.4	1.6		
	LC	0.0	0.0			0.0	0.0	0.0	0.0		
	D	0.0	0.0			0.0	0.0	0.0	0.0		
2	N	93.3	5.8	95.1	4.6	90.6	9.1	88.6	6.4	98.0	3.4
	NP	5.0	5.0	1.5	2.6	7.9	7.1	0.0	0.0	0.0	0.0
	AN	1.7	2.9	1.9	3.2	1.5	2.6	11.4	6.4	2.0	3.4
	LC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	D	0.0	0.0	1.5	2.6	0.0	0.0	0.0	0.0	0.0	0.0
3	N	86.9	7.4	93.7	1.1	57.6	16.8	96.3	3.2	92.7	0.3
	NP	11.3	7.0	2.4	5.1	47.3	10.8	0.0	0.0	0.0	0.0
	AN	1.8	3.0	1.9	3.9	3.7	6.4	3.7	3.2	7.3	0.3
	LC	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	D	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	N	73.7	8.0	74.8	13.5	82.0	5.9	89.1	12.8	95.2	8.2
	NP	22.6	11.0	12.4	6.8	10.0	3.6	5.6	9.6	2.4	4.1
	AN	0.0	0.0	0.0	0.0	8.0	3.3	5.3	4.6	2.4	4.1
	LC	1.7	2.9	12.9	14.5	0.0	0.0	0.0	0.0	0.0	0.0
	D	2.0	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	N	22.7	21.1			94.0	6.3	91.7	9.2		
	NP	19.4	33.7			2.1	3.6	5.3	4.7		
	AN	24.2	21.0			3.9	3.4	3.0	5.2		
	LC	33.7	32.3			0.0	0.0	0.0	0.0		
	D	0.0	0.0			0.0	0.0	0.0	0.0		

In comparing the two studies, the previous study did not account for any loss of suspension so the concentrations presented were likely higher than the experimental conditions. The current study model generated LC50 values of 112 mg l^{-1} for eight-day larvae and 380 mg l^{-1} for 21-day larvae, which is the reverse of the trend in the earlier work. The difference in ages of larvae between the two studies may influence the results, as could differences in drill cutting source. Another explanation for the apparent contradiction is the decrease in model accuracy when predicting values higher than the experimental concentrations. The confidence intervals of the eight-day and 21-day LC models overlap at all concentrations (Fig. 8F), and become more extreme at the higher concentrations, indicating high uncertainty in the model. The studies do concur that severe effects on *L. pertusa* larvae occurred at drill cutting concentrations >280 mg l^{-1}.

Bentonite was the only material that showed lethal effects within the experimental concentration range; the LC50 for the 21-day larvae was 53 mg l^{-1}. The LC50 concentrations for the other materials and larval ages were extrapolated from the statistical model rather than derived experimentally. More experimental work should be done to validate the statistical models, but given the different physical properties of these materials, it is not surprising that they elicited different responses during the larval experiments.

Bentonite is comprised of very fine particles that form a colloid in solution, barite is a very heavy crystalline material that is used as a weighting agent, and drill cuttings are a mixture of bedrock and drilling fluids, so will vary in composition (e.g. Sanzone et al., 2016). The high viscosity of bentonite may affect the cilial movement and therefore the larval ability to swim. The swimming speed was not a quantified experimental metric but general observations on swimming behavior were made during larval examination. Reduced swimming speed was observed at lower concentrations in bentonite than either of the other materials. Reduction in swimming speed was not observed in the barite experiment until the highest concentration, when most of the larvae had particles attached and some were completely clogged. Larval swimming in the drill cuttings experiment began to slow down at mid-range concentrations. Although recovery of the affected larvae was high under experimental conditions, in a field situation, larvae that cannot swim well may sink to the seafloor or be easily preyed upon. Factors that impact swimming speed, vertical migration, and the ability of larvae to remain suspended in the water column, may reduce larval dispersal potential (Strömberg and Larsson, 2017), and ultimately connectivity among reefs. Additionally, slower ciliary movement can affect feeding efficiency, causing the larvae to ingest less food (Strömgren and Larsson, 2017).

The current study shows age-related effects for bentonite and drill cuttings, although the threshold concentrations for severe impacts differed between the materials. At higher concentrations, the colloidal bentonite particles can clog the longer, denser cilia of the older larvae and slow down swimming. The sparse cilia of the younger larvae may expose the delicate epithelium to sharp drill cuttings.

As discharge from drilling is ejected in pulses, there may be a possibility for the larvae to recover, wholly or partly, in between the pulses. As far as the authors are aware, there have not been any studies investigating the potential recovery of cold-water coral larvae after suspended particle exposure. In all concentrations of barite and drill cuttings, >82% had recovered seemingly fully while in the highest
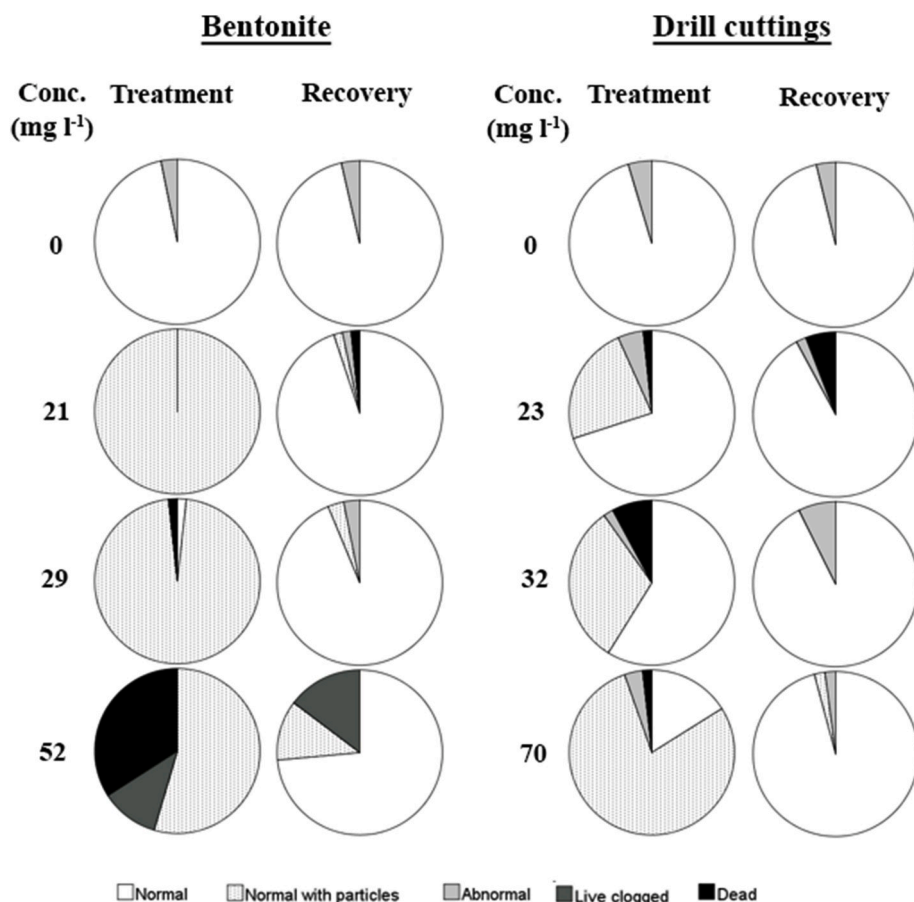
Fig. 7. Treatment and recovery effects (% of each response category) of three test materials on 21-day larvae. Both treatment and recovery period lasted 24 h. Each pair diagram represents response after exposure and recovery from a single concentration. Response categories: N = normal, NP = normal with particles, AN = abnormal, LC = live clogged, D = dead.

concentrations of bentonite the recoveries were not as successful. It appears that if a larva is clogged by bentonite it cannot recover as it is incapable of freeing itself of the mucus capsule. The results of the recovery phase are interesting as they imply that the pulse-wise discharge during drilling operations works in favor of the larvae. If the larvae are not clogged with particles, this potentially gives them an opportunity to rid themselves of particles caught in the cilia. In our experiments, the recovery phase was 24 h while the time between discharge pulses during drilling often are hours. Studies on recovery periods that more closely mimic industry operations will provide further insight into their effects on larval populations.

During exploratory drilling at Morvin A in the Norwegian Sea, nine *L. pertusa* reefs close ($\geq$100 m) to the discharge point, were monitored for four months during drilling operations. Particle concentration models were developed to assess the effects of drilling discharges on the nearby reefs (Purser, 2015). Maximum measured discharge concentration was 80.1 mg l^{-1}, but according to the study, the reefs were probably not exposed to concentrations >25 mg l^{-1} for more than a few days in total. The study documented no changes in coral behavior, reef structure or associated community structure (Purser, 2015). However, the results of this study show that almost 50% of larvae could be affected (EC50 38–40 mg l^{-1}) if drilling was undertaken while coral larvae were present. *Lophelia pertusa* larvae may therefore be at risk from drilling operations, if they occur during the coral reproductive period.

The spawning period of *L. pertusa* covers a period of approximately 3 months from January to March (Brooke and Järnegren, 2013). During this extended time period, *L. pertusa* colonies release eggs and sperm directly into the water column, where they fertilize and develop into swimming larvae after approximately 3 days (Larsson et al., 2014). The

effects of sediment on deep sea coral gametes is unknown but work on shallow water tropical species has shown that 'realistic' levels of sediment can severely reduce fertilization success (Ricardo et al., 2015, 2016a). Some species of tropical corals release buoyant bundles of gametes, which float to the surface where they break apart and fertilization occurs. Low to moderate levels (35–87 mg l^{-1}) of suspended sediment can prevent these gamete bundles from reaching the surface, thereby reducing egg-sperm encounter rates and compromising fertilization success (Ricardo et al., 2016a). A related study has shown that suspended sediment can entangle and sink coral sperm, reducing concentrations by up to 45%, and reducing fertilization rates (Richardo et al., 2015). After fertilization, embryos are passive particles in the water column until they develop cilia. Ricardo et al. (2016b) found no reduction in survival or successful metamorphosis in coral embryos exposed to sediment concentrations up to 1000 mg l^{-1}; however ~ 10% of the embryos became enveloped in mucous/sediment coating and sank. These embryos developed normally when transferred to clean water and once ciliated were able to break free of their cocoon. However, in a natural setting, an embryo that sinks to the seafloor would be vulnerable to suffocation, predation or other hazards.

Larvae of *L. pertusa* may remain in the water column for 3 weeks or more (Larsson et al., 2014), during which time, they may be exposed to natural or anthropogenic suspended sediment or contaminants. Particle deposition can also prevent coral larval settlement, and smother newly settled juveniles (Hodgson, 1990; Gilmour, 1999; Babcock and Smith, 2002; Perez et al., 2014). These combined effects on larvae and juveniles may have large implications for the recruitment of new corals into the population. Given the potential for *L. pertusa* larvae to disperse over large distances (Larsson et al., 2014; Strömberg and Larsson, 2017), the
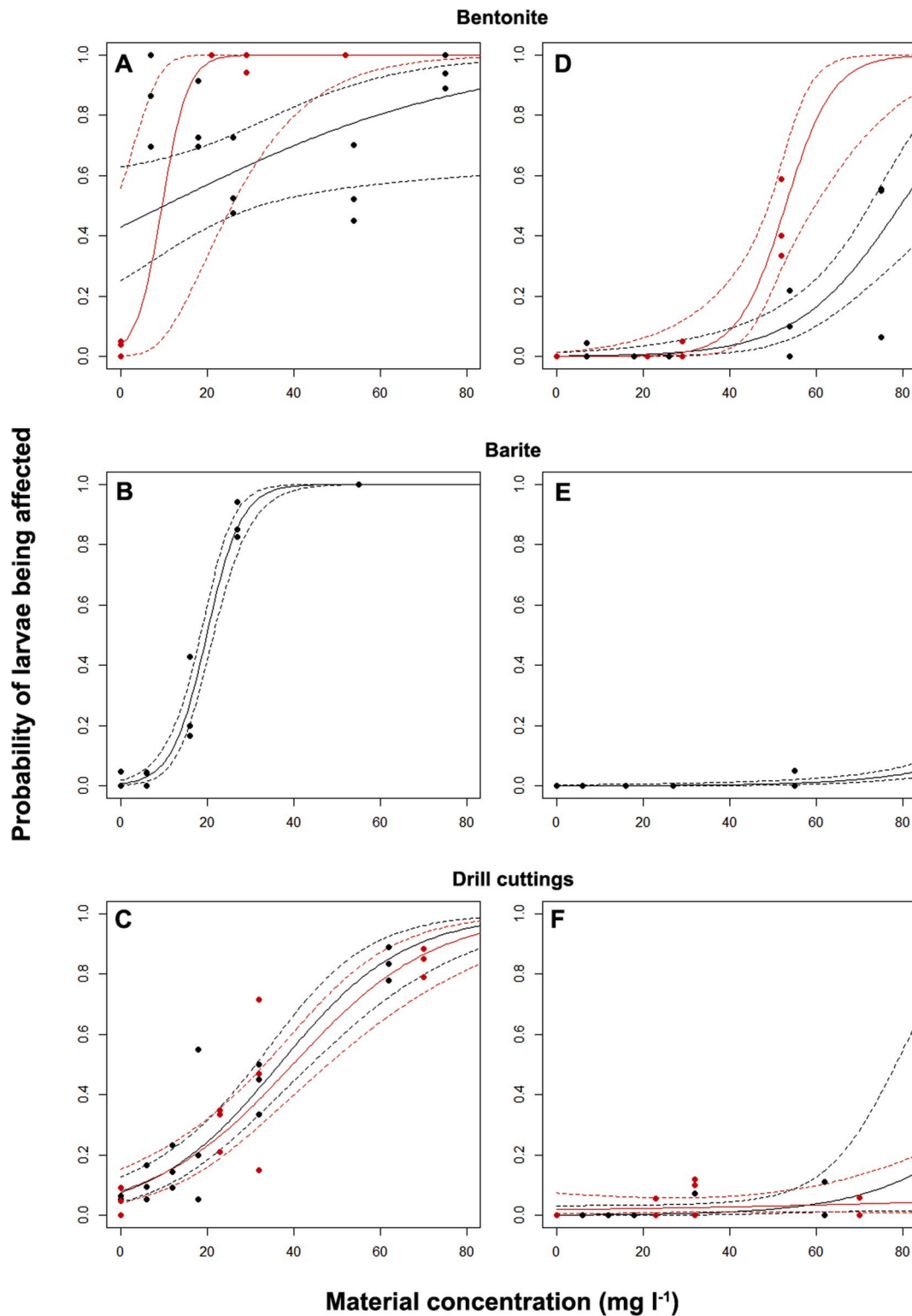
Fig. 8. Logistic regression models for eight-day (black line) and 21-day (red line) old larvae showing affected larvae of two cumulative categories 'all effects' = all categories except normal, and 'severe effects' = live clogged or dead. Left side panels are models for 'all effects': A) bentonite, B) barite, C) drill cuttings. Right side panels are models for 'severe effects': D) bentonite, E) barite, F) drill cuttings. Experiments with barite were only carried out on eight-day larvae. Y-axis is percentage of larvae affected while x-axis is material concentration (mg l^{-1}). For more details see Table S1. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

consequences of impacting recruitment are likely to occur beyond the immediate discharge area.

Understanding how early life history stages are affected by concentrations of different suspended particulates can help inform

management strategies to protect marine ecosystems for human activities. The risk of affecting *L. pertusa* reproductive success and recruitment could be lessened by temporal management; for example, restricting drilling in areas with known cold-water corals during their

Table 4

EC = effect concentration. Concentration where 10, 20 and 50% of the larvae are in some way affected by the material (Normal with particles or worse). LC = lethal concentration. Concentration where 10, 20 and 50% of the larvae are live clogged or dead.

	Bentonite		Barite	Drill cuttings	
	8-day	21-day	8-day	8-day	21-day
EC10 (mg l-1)	0.0	3.1	11.2	4.5	3.8
EC20 (mg l-1)	0.0	5.5	14.4	16.4	17.1
EC50 (mg l-1)	10.1	9.6	19.9	37.7	39.8
LC10 (mg l-1)	53.3	40.9	96.9	77.0	170.1
LC20 (mg l-1)	62.9	45.3	110.3	90.1	247.6
LC50 (mg l-1)	79.5	53.0	133.4	112.4	380.1

reproductive period, would minimize impacts on early life history stages. This is particularly important for drilling materials, such as bentonite, that have more severe impacts. If drilling is permitted during the reproductive period, longer periods between the discharge pulses could provide the larvae opportunity to rid themselves of particles, with the exception of bentonite which they cannot remove. Minimizing the amount of bentonite (and other such materials) used in drilling operations would also reduce the environmental risk to larvae of corals and other species.

The use and discharge of water based chemicals has given a larger spread of drilling mud material in the form of suspended particles. But there is a considerable lack of data on the effects of suspended particles on marine organisms in general. There is little information about effects on larvae and even less on cold-water corals and other deep-sea species. The results from this study provide some insight into the effects and recovery potential of larvae of a single keystone species; however much more work is needed to understand the reproductive periods and larval sensitivities of other reef fauna. Such data would support Coral Risk Assessment models and contribute to further refinement of models used as decision support tools for management of operational discharges to the marine environment, for example the DREAM-model (Reed and Rye, 2011; Ulfsnes et al., 2012).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Johanna Järnegren: Conceptualization, Investigation, Methodology, Resources, Project administration, Funding acquisition, Writing - original draft. **Sandra Brooke:** Conceptualization, Investigation, Methodology, Writing - original draft. **Henrik Jensen:** Methodology, Formal analysis, Writing - original draft.

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Appendix A. Supplementary data

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