Acute and Physical Effects of Water Based Drilling Mud in the Marine Copepod *Calanus finmarchicus*

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Running head: **Effects of drilling mud on copepods**

Abstract

In this study we investigated impacts of the fine particulate fraction of a commonly used barite–containing drilling mud on the pelagic filter feeding copepod *Calanus finmarchicus*. The results show that the tested drilling mud had a low acute toxicity on *C. finmarchicus* (LC_{50} > 320 mg/L) and that the observed toxicity most likely was caused by dissolved constituents in the mud and not the particle phase containing the weighting agent barite. Further, animals were exposed to drilling mud at a concentration of 10 mg/L for 168 h followed by a 100 h recovery phase. A rapid uptake of drilling mud particles was observed while the excretion was slow and uncomplete even after 100 h recovery in clean seawater. The uptake of drilling mud particles caused a significant increase in sinking velocity of copepods, indicating that uptake of drilling mud particles affected their buoyancy. Long term exposure to low concentrations of drilling mud could therefore cause physical effects such as impacts on the animals' buoyancy which may affect the energy budget of the copepods.

Key words

Water based drilling mud, barite, *Calanus finmarchicus*, acute toxicity, uptake, sublethal effects, buoyancy

Introduction

The offshore oil industry discharges drilling mud and drill cuttings, which derive from exploration and drilling activities, into the sea. Drilling muds contain mostly natural or modified clays, polymers and weighting materials and in smaller amounts solids or liquid chemicals suspended in a base fluid. The drilling mud serves several purposes, i.e. to transport drill cuttings to the surface, balance subsurface and formation pressures preventing blowouts, and to cool, lubricate and support parts of the drill pipe (Sanzone et al., 2016; Neff, 2008). Drilling muds can be classed as water-based (WBM), oil-based (OBM) or synthetic-based (SBM) depending on their base fluid. For most offshore oil drilling activities on the Norwegian continental shelf WBMs are used, due their lower environmental impacts compared to OBMs and SBMs. In European offshore waters WBM may be permitted for ocean discharge (OSPAR 2000). Barite (barium sulphate) is one of the most frequently used weighting materials in WBM because of its low water solubility and high density (Burton et al., 1968).

The use of barite containing WBM drilling mud is considered as environmentally safe, since it is not reported to be acutely toxicity for marine organisms and exhibits a very low bioaccumulation factor due to a low solubility in seawater (Burton et al., 1968; Neff et al., 1989a; Neff et al., 1989b). However due to the small size of barite particles (10-20 µm) used in drilling mud formulations, and the presence of mineral impurities (silica, iron oxide and certain heavy metals; Neff, 2008), effects on filter feeding organisms may occur. Previous experiments showed altered survival and growth in sea scallops (*Placopecten magellanicus*) even at low concentrations of WBM (effect limit 10 mg/L) and pure barite (effect limit 0.5 mg/L) (Cranford et al., 1999). Adverse effects such as reduced filtration rates, reduced growth or evidence of shortening, coagulation and disintegration of gill structures following barite and used WBM exposure were reported in the filter feeding bivalves *Cerastoderma edule, Mytilus edulis* and *Pecten maximus* (Barlow and Kingston, 2001; Bechmann et al., 2006). Further, histopathological changes of gills were reported in cod (*Gadus morhua*) exposed to used WBM and barite (Bechmann et al., 2006).

The pelagic filter feeding copepod *Calanus finmarchicus* represents by far the most abundant copepod species and dominates the zooplankton biomass in Norwegian waters and the North Atlantic (Sakshaug et al., 1992). It has a wide geographical distribution and high annual production. *Calanus finmarchicus* is an effective filter feeder foraging mostly on phytoplankton during the spring bloom and during summer, while late copepodites overwinter in diapause at a few hundred meters depth. *Calanus finmarchicus* is undergoing regular diurnal vertical migrations in the water column and their buoyancy in the water is therefore likely highly controlled (Irigoien, 2004). The uptake of weighting particles such as barite could impact the animal's ability to control their buoyancy.

In this study we investigated the impacts of the fine particulate fraction (<50 µm) of the commonly used barite-containing WBM Glydril™ on adult *C. finmarchicus*. The fine particulate fraction of drilling mud is assumed to be the most relevant regarding impacts on pelagic copepod species, since larger particles will rapidly sediment out of the water column after release, and the smaller particles might be in the size range of natural food particles for the copepods (Hebert and Poulet, 1980; Nejstgaard et al., 1995; Båmstedt et al., 1999).

The main objectives of the current study were to i) investigate the acute toxicity of drilling mud fine particulates (<50 µm), ii) study whether *C. finmarchicus* filters drilling mud particles including barite (bioavailability), and iii) determine if weighting particles can affect the buoyancy of copepods.

Materials and Methods

Exposure preparation and characterisation

Elemental analysis of drilling mud

In order to verify the presence of barite in drilling mud samples drilling mud was analyzed with X-ray fluorescence (XRF) and X-ray diffraction (XRD). Drilling mud was milled manually and dried for 3 days at 60 °C. Samples were prepared by adding 0.5 g of the dry material to 5 g flux (lithium tetraborate 66%; lithium metaborate 34%), with a subsequent addition of 60 µl lithiumiodite. The mixture was melted to form tablets. Tablets were analyzed directly for main elements. For trace element analysis 8 g of dried samples were mixed with 2 ml Elvacite® (Lucite International, USA), thereafter and tablets were pressed. The tablets were analyzed directly with a PW1480 instrument (softwareX40; Phillips, Netherlands). For XRD analysis samples were pressed without further additions and analyzed (PW 1830, Phillips, Netherlands) with settings described in SI table 1.

Preparation of stock dispersions

In order to prepare drilling mud dispersions with a particulate fraction <50 µm, drilling mud (27 g L^{-1}) was thoroughly mixed with seawater in a 2 L glass bottle and left to settle for 6 min to remove larger particles (>50 µm). The supernatant was decanted and used as stock dispersion in the exposure studies. The removal of large particles was verified applying laser scattering measurements with a LISST-100X (Sequoia Inc., USA) measurements (Supporting Information: Figure S1). The dry mass of the stock dispersion was determined after repeated washing steps (MilliQ water) and centrifugation (Hettich Universal 32K, Hettich, Germany), followed by drying at 60 °C for 24 h. The exposure dispersions were prepared as dilutions based on the determined dry weight.

Particle shape

The shape and size of particles present in prepared drilling mud exposures was investigated by phase contrast light microscopy (Nikon eclipse 80i; 20x Plan-Fluor Ph1DLL 0.5NA objective; Nikon, Japan).

Acute exposures

Organisms

Calanus finmarchicus (Gunnerus) from a permanent laboratory culture, which was initially established from stage V copepodites collected in Trondheimsfjorden, Norway, were used as test organisms. The culture is routinely kept at 10 °C. Details regarding the culturing conditions are described in Hansen et al. (2007).

Acute toxicity assessment

We determined the acute toxicity of the "total" drilling mud (dissolved and fine particulate fraction) and the dissolved constituents present in the drilling mud (dissolved fraction only). Exposure dispersions were prepared from stock dispersions generated as described above. For total drilling mud exposures, the stock dispersions were diluted to the tested concentrations (see below) with filtered natural seawater and well mixed. In order to remove the particulate fraction for dissolved fraction exposures, the stock dispersion was centrifuged at 2000 rpm (Hettich Universal 32 R) for 5 min. Samples were analysed with a LISST-100X to confirm the removal of particles (Supporting Information: Figure S1). The obtained dispersion was diluted with filtered seawater in the same ratios as the particle exposures to obtain comparable exposure concentrations regarding the dissolved fraction.

Adult copepods from the continuous culture were exposed for 96 h to total drilling mud and the dissolved fraction at the following concentrations: 5, 10, 20, 40, 80, 160 and 320 mg/L. Each concentration and condition was tested in triplicate (*n*=3), except for negative controls (clean seawater, *n*=6), with 7 animals present in each container (500 mL). The exposure bottles were gently agitated twice a day to resuspend settled particles. The test animals were not fed during exposure, and the exposure solutions were not renewed. Animal survival was assessed daily over the 96 h exposure period.

Uptake and sublethal effects

Sublethal exposure

The experimental setup is shown in Figure 1. A polyethylene tank with 45 L filtered sea water was used to expose the animals to 10 mg/L of the fine particulate fraction of the drilling mud. The stock suspension of the drilling mud was prepared as described above, and fed to the water flow to the exposure tank by a tubing pump (Watson Marlow 202, England) at a rate to give 10 mg/L final exposure concentration.

14 hours prior to onset of the exposure, approximately 1200 adult copepods were transferred into the exposure tank. At the time of the exposure onset (time point 0), control copepods were sampled (group C). Following, copepods were sampled at four different time points during the exposure: 14 h (group E1), 38 h (group E2), 90 h (group E3) and 168 h (group E4). After 168 h, the exposure was terminated and the remaining copepods were transferred to a new tank containing clean seawater for recovery. Subsequently, copepods were sampled at three points in time during the recovery period: 24 h (group R1), 48 h (group R2) and 100 h (group R3). During the whole exposure and recovery period the copepods were fed with the unicellular algae *Dunaliella tertiolecta* at a concentration of 2.5 million algae/L/d.

Uptake of drilling mud

To determine the uptake of drilling mud particles, copepods (*n*=10) were sampled at each of the time points described above, weighed, frozen, lyophilized and reweighed. Due to the small sample size of individual copepods, the sampled individuals were pooled for analysis. The samples were digested in 0.5 ml ultrapure HNO_3 at 110 °C for 1.5 h. Subsequently the samples were diluted with MilliQ water to a total volume of 12 ml, and analysed for selected elements using inductively coupled plasma mass spectrometry (ICP-MS, Element 2; Thermo Finnigan, USA) as described in more detail elsewhere (Sørmo et al. 2011) at the core facility of the Department of Chemistry, NTNU (Trondheim, Norway).

To study the uptake of drilling mud particles microscopically, copepods (*n*=96) were sampled at each time point described above. Copepods were removed carefully from the exposure tanks and randomly divided into 4 groups with 24 animals each. The animals were then irreversibly sedated with MS-222 (Finquel, Argent Chemical Laboratories, USA) by adding a 750 mg/L stock solution drop wise in a petri dish containing the copepods and sea water until cessation of swimming activity. Images were captured with a dissecting microscope (MZ125; Leica Microsystems, Germany) equipped with a CCD camera (DFW-SX900; Sony Cooperation, Japan). The images were used to assess the content of mud particles in the animal's digestive tract as well as for the analysis of lipid storage for groups C (control) and E1 (14 h exposure) using a protocol described in Miller et al. (1998) and previously used in our laboratory (Hansen et al., 2008). The size of the lipid storage was determined as projected 2D lipid area (mm^2) measured on the scaled captured images using the imaging software ImageJ (National Institute of Health, USA).

Sinking velocity determination

The sinking velocity of individual copepods (*n*=96) was determined at all sampling time points described above by measuring the time the anesthetised copepods (MS-222) need to sink a distance of 24 cm in sea water. Measurements were taken in a 1 L glass cylinder. Sinking rates were calculated as mm sinking/sec.

Statistics

Data analyses were performed with GraphPad Prism 7 (GraphPad Software Inc., USA). The data sets were analysed for normality (Shapiro-Wilk normality test) and homogeneity of variance Bartlett's test. To detect significant differences between treatments data were analysed either with ANOVA followed by Tukey's multiple comparisons test or with the non-parametric Kruskal-Wallis statistics followed by Dunn's test. Linear regression analysis was applied in order to analyse correlations between lipid-content and sinking speed.

Results

Drilling mud characteristics

X-ray fluorescence and XRD analyses showed that the drilling mud samples contained barite (Supporting Information: Figure S2; Table S2). The barium (Ba) concentration was 64 mg/g dry mass (XRF), resembling approximately 109 mg/g barite in the drilling mud sample. Microscopic images showed that the prepared fine particulate fraction of drilling mud contained mostly small particles that were predominantly roundish or slightly edged in shape (Supporting Information: Figure S3).

Acute toxicity

The acute toxicity of drilling mud was assessed in the presence and absence of particles in order to determine whether the particulate fraction or the dissolved components caused an effect. The results showed an acute toxic effect (40 % lethality, Figure 2A) occurred only at the highest drilling mud concentration (320 mg/L). Similarly, an acute toxicity (lethality 35 %; Figure 2B) occurred at the highest exposure concentration (320 mg/L) in the dispersions in which the particulate fraction has been removed. An acute LC50 concentration could not be determined from either experiment, because the highest exposure concentrations caused <50 % lethality.

Uptake and sublethal effects

Uptake of drilling mud

Figures 3B-C show uptake of drilling mud particles during the experiment. The images show that the copepods filtered algae and drilling mud particles, as green algae are visible in the copepods digestive system in the control group (Figure 3A), while dark coloured particles are visible in the exposed copepods (Figure 3B+C). After 14 h of exposure, 38±6% of the animals had dark coloured particles in their guts. This increased significantly ($p=0.035$) to 64±5 % after 168 h of exposure (Figure S4). After the 100 h recovery phase the dark colouration within the stomach and digestive tract area was still visible (Figure 3D), however in significantly fewer animals ($p=0.003$) than at 168 h (30±6%, Figure S4).

Uptake of drilling mud was further assessed through analyses of drilling mud constituents Ba, Si and Al with ICP-MS. Concentrations of Ba in the analysed animals increased from 0.27 µg/g dry weight in controls to 98 µg/g after 14 h of exposure, and reached a maximum concentration of 353 µg/g after 90 h of exposure (Figure 4A). At 168 h, concentrations were slightly lower with 226 µg/g. Ba concentrations decreased in the recovery period, but remained above 100 µg/g even after 100 h of recovery. Similarly, Al and Si concentrations increased during exposure. Al concentration in controls was 1.7 µg/g dry weight, increased steadily to 24.7 µg/g, and declined to 8.4 µg/g after a 100 h recovery period (Figure 4B). Control Si concentrations were 17.7 µg/g dry weight, reaching 46.8 µg/g after 168 h exposure, and declined to 29.7 µg/g during recovery (Figure 4C).

Sinking velocity

In the exposed group, a steady increase in sinking velocity was observed throughout the experimental period (Figure 5). At the 90 h exposure time point the sinking velocity increased significantly ($p=0.002$) by approximately 1 mm/s compared to control animals and remained significantly higher ($p<0.01$) throughout the exposure and recovery period (Figure 5).

The projected 2D lipid sack area was determined from microscopic images in the control and 14 h exposure group. While a significant (inverse) correlation between the lipid storage area and the sinking velocity was found in the control groups ($p<0.0001$; $R^2=0.322$), no significant correlations ($p>0.05$; $R^2= 0.0392$) were detected in the exposed group (Figure 6).

Discussion

In the 96 h acute exposure experiment lethality was only observed at the highest tested concentration (320 mg/L) and no LC_{50} concentration could be determined for the fine particulate fraction of the drilling mud tested in this study. A similar toxicity level, i.e. lethality occurring only in the highest exposure concentration, was observed in the acute exposure to drilling mud samples after removal of the particulate fraction. This shows that water soluble residues were the likely cause of the observed toxicity. It was previously shown that a KCl-polymer mud was the most toxic of 8 tested generic WBMs (USEPA, 1985). X-ray diffraction analysis showed considerable amounts of K2O in the used drilling mud applied in this study, potentially deriving from KCl in the mud. Barite constituted approximately 10 % of the drilling mud dry mass in the present study. However, barite is almost insoluble in water and present as fine particles in WBM, and is thus likely not contributing to the lethality observed in this study. Our results are in agreement with previous studies reporting limited acute toxicity of WBM and barite in various marine species including copepods (for review see Smit et al., 2006).

While the tested drilling mud samples showed acute effects only at high exposure concentrations, *C. finmarchicus* was found to accumulate drilling mud particles also at relatively low exposure concentrations (10 mg/L). Filtering and uptake of drilling mud particles into the digestive system was observed already after 14 h of exposure. Further, the presence of drilling mud remains in the digestive tract during the recovery period indicates incomplete removal even after termination of the exposure. Uptake of drilling mud was also shown by increased concentrations of the selected marker elements Ba, Si and Al in exposed animals. Concentrations of Si and Al increased continuously during exposure, while a slight decrease of Ba was observed in the last exposure time point, however, this can likely be attributed to uncertainties resulting from the small sample size. Concentrations of Ba, Al and Si declined during the recovery period, but remained above control levels even after 100 h recovery in clean sea water. This was pronounced especially for Ba, where recovery levels were still around 500 times higher in exposed animals compared to control animals. Uptake of Ba from barite-spiked sediments was previously shown for the benthic organisms *Nereis diversicolor* and *Hinia reticulata* (Schaanning et al., (2002). Further, previous studies reported unselective feeding behaviour of *C. finmarchicus* and showed the uptake of inorganic particles such as natural sediments and released mine tailing particles (Arendt et al. 2011; Farkas et al. 2017) and even uptake of particulate oil droplets when exposed to oil dispersions (Hansen et al., 2012). Systemic bioavailability of elements and toxic heavy metals, which can be present in particulate form or bound to drilling mud will be dependent on the solubility within the copepods digestive system. The pH in the stomach of *Calanus helgolandicus* was shown to be lower (6.86 - 7.19) compared to sea water (Pond et al. 1995). A previous study reported leaching of Cd, Cu, Pb and Zn from barite at acidic conditions, simulating the gut of deposit feeding benthic animals (Crecelius et al., 2007). However, Si, Al and Ba will likely not dissolve and mostly remain as particles in the animals guts. It was previously shown that less than 1 percent of Ba were soluble within 48 h under acidic conditions mimicking a copepods gut (Crecelius et al., 2007).

Beyond elemental toxicity, the ingestion of inorganic particles can potentially lead to physical impacts in copepods. Here we assessed the effect of drilling mud uptake on the buoyancy of copepods and showed that the sinking velocity of exposed individuals significantly increased compared to unexposed individuals. Increased sinking velocity was observed in all exposures from 90 h onward. This is in agreement with a previous study of Shadrin and Litvinchuk (2005), who reported impacts on the locomotion and increased sinking velocities in the copepod *Acartia clausi* after the exposure to inorganic mineral particles.

In our study we found that the sinking velocity remained elevated also throughout the recovery period, which is in agreement with our observations of drilling mud in the copepods digestive system. However, element concentrations and the relative number of animals containing dark coloured particles in their guts were reduced in copepods during the recovery phase. The continuing increased sinking velocity despite decreased element concentrations during recovery phase could pinpoint towards a lowered energy availability and subsequent increasing usage of stored lipids to meet necessary energy demands, as the ingestion of inorganic particles was shown to result in reduced energy intake (Paffenhöfer, 1972; Shadrin and Litvinchuk, 2005). Suggestions have been made that the buoyancy is also controlled by the lipid storage size of copepods (Irigoien, 2004). *Calanus finmarchicus* develops a lipid reservoir during the last three copepodite stages (CIII-CV) which is ultimately used for production of eggs and spermatophores during adulthood (Caspers, Marshall, and Orr 1973). Therefore, the size of the lipid storage may be varying at the adult stage between 20-50% of total body volume (Hansen et al., 2008). It is expected that copepods with larger lipid reservoir will float better than copepods with a small lipid reservoir. In this work we analysed the lipid storage represented as projected 2D area of the control and the 14 h exposure group. Results show that there was a significant inverse relationship between these two parameters in the controls, which was absent in the 14 h exposed animals. This may be explained by the ingestion of weighting particles including barite, which was observed already after 14 h of exposure. This is providing further evidence for the proposed effects of drilling mud on buoyancy. However, further studies investigating the relationships between uptake of weighting particles, buoyancy and energy uptake and demands over extended exposure periods are needed.

The main conclusions from this study are that the fine particulate fraction of the water based drilling mud used in this study caused acute toxicity in *C. finmarchicus* only at relatively high concentrations. However, *C. finmarchicus* was found to filter the fine particles, which are retained in their digestive system over extended time periods. The uptake of weighing particles can affect the copepods density as indicated by their sinking velocity and could thus have effects on their energy budget, fitness and survival under chronic exposure. Copepods such as *C. finmarchicus* are a key component in the pelagic food chain in the North Atlantic and North Sea, thus further research is needed to investigate sublethal impacts of drilling mud uptake such as energy budget disturbance.

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Figures

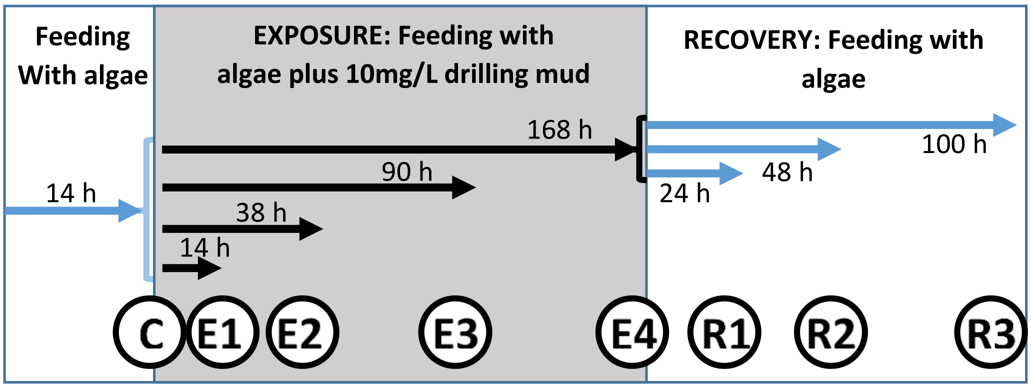

Fig. 1: The setup of the main experiment. Copepods were transferred to a tank and fed for 14 h prior to exposure. At the time of exposure initiation, control groups were sampled, and during the exposure copepods were sampled four times. Thereafter the remaining copepods were transferred to clean water for recovery, and during this recovery period three samplings were conducted. Circles in the lower section indicate the notation used for the different sampling times.

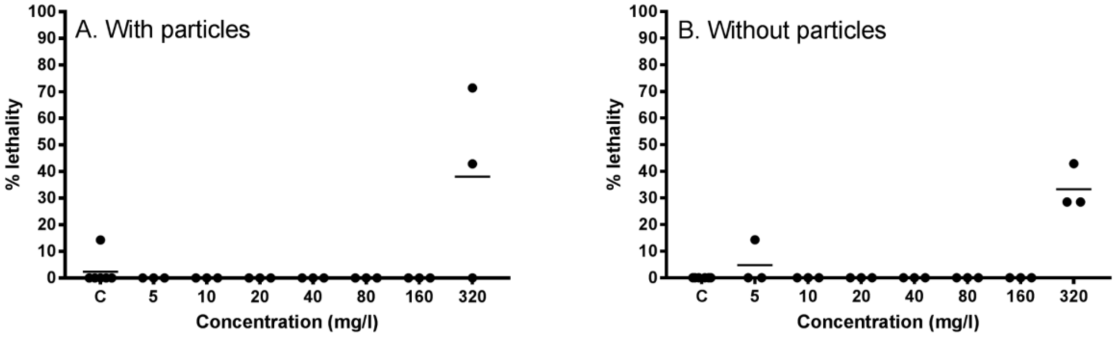

Fig. 2: Acute toxicity of drilling mud before (A) and after (B) centrifugation of the stock solution. A: Drilling mud with particles. B: Centrifuged drilling mud. For each concentration dots represent replicate groups and horizontal line segments the mean value.

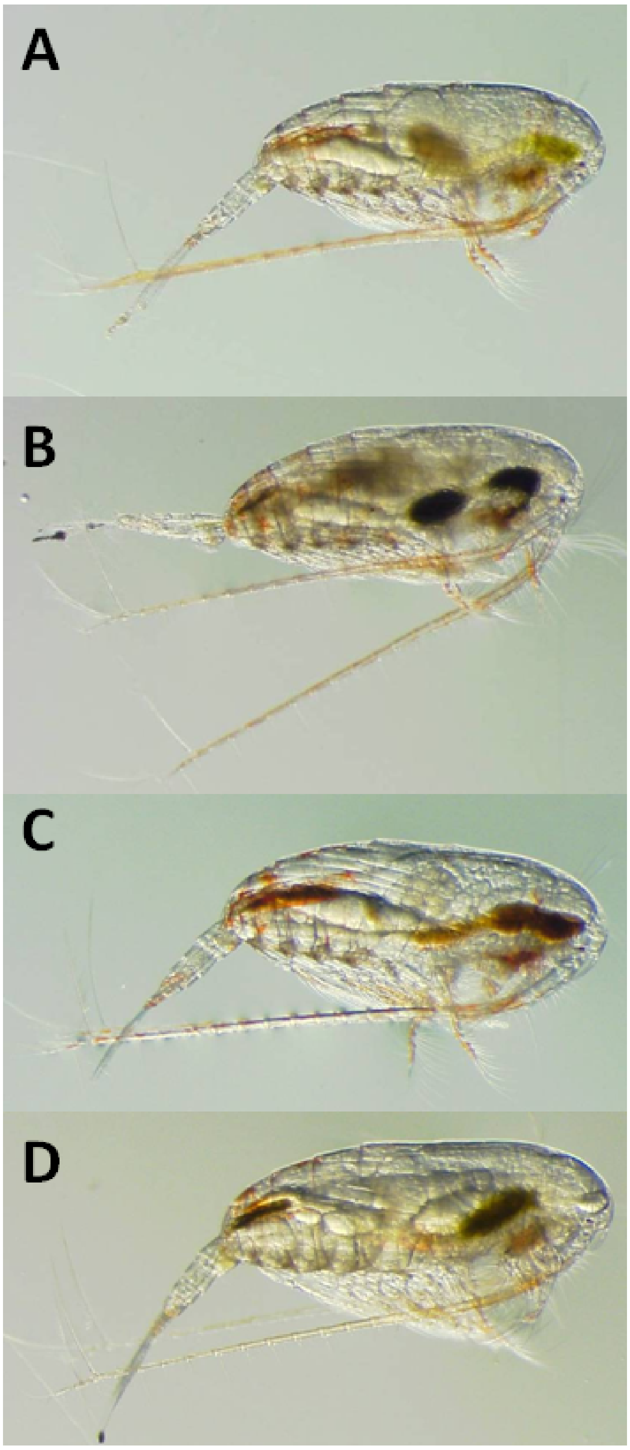

Fig. 3: Representative microscopic pictures of copepods. A. Control copepods fed only with algae (C). B+C. Copepods exposed to drilling mud for 14 h (E1). D. Copepod exposed to drilling mud for 168 h and recovery for 100 h (R3).

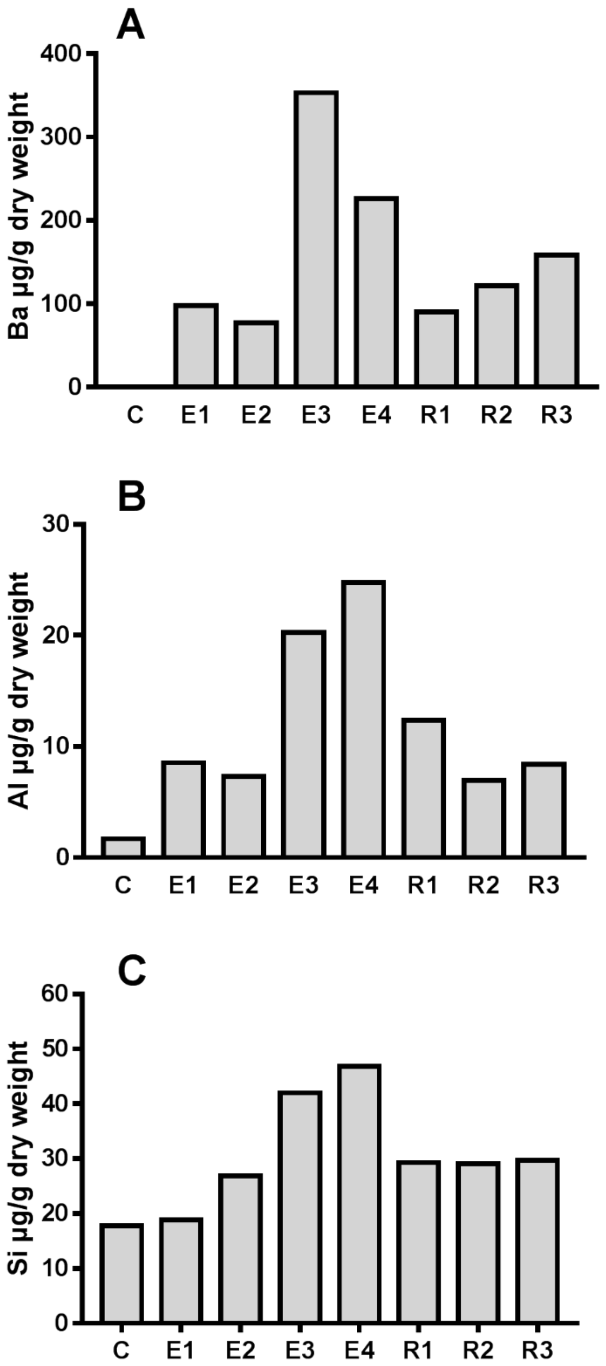

Fig. 4: Concentrations of barium (A), aluminium (B) and silica (C) in copepods (µg/g tissue dry weight) exposed to 10 mg/L drilling mud. Each bar represents the average metal concentration in pooled copepod samples (*n*=10). Names of the exposure groups refer to Figure 1.

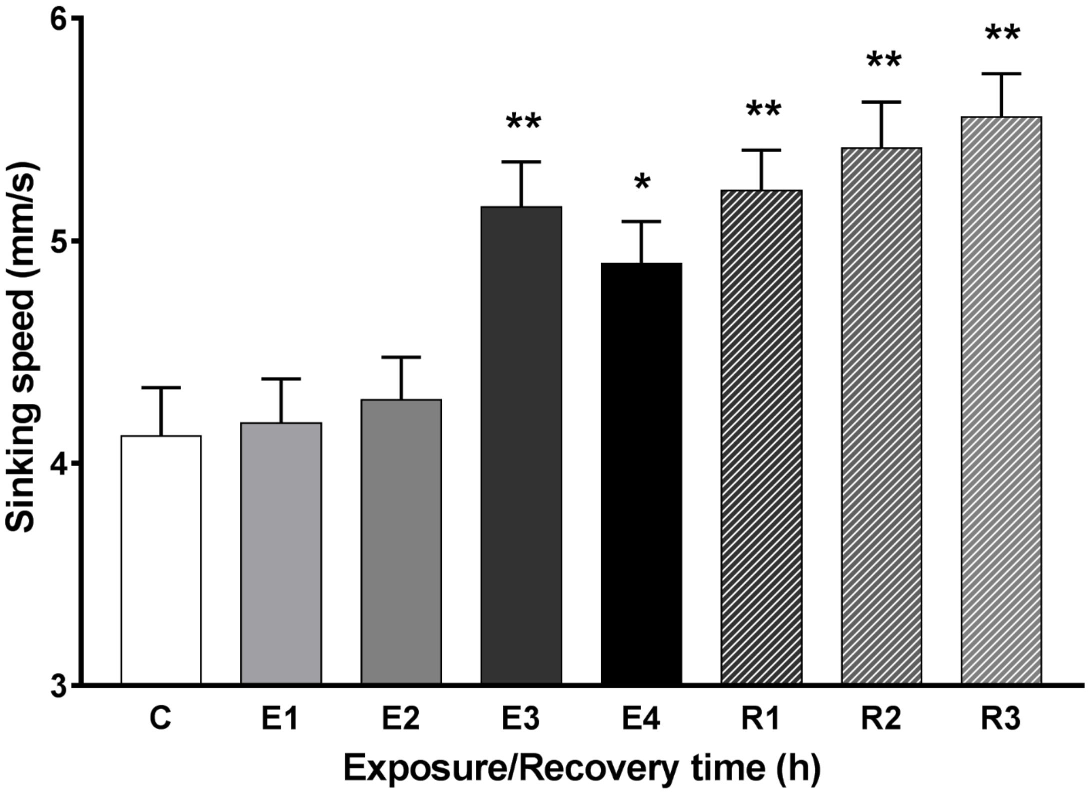

Fig. 5: Sinking velocity (in mm/sec) measured for individual sedated copepods. C; controls. E1 – E4; exposure to 10 mg/l drilling mud for 14, 38, 90 and 168 h. R1 – R3; Recovery in clean seawater for 24, 48 and 96 h after exposure to 10 mg/L drilling mud for 168 h. Data are presented as mean±SE; *n*=96. Significant differences compared to the control group are indicated as * ($p<0.05$) and ** ($p<0.01$).

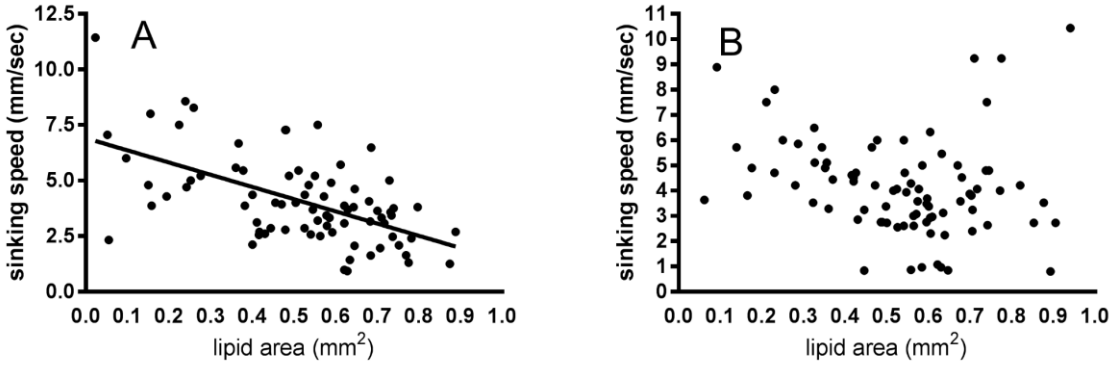

Fig. 6: Sinking velocity (mm/sec) as a function of lipid storage size (measured as lipid area in mm^2) for the controls (C; n=78) and the 14 h exposed group (E1; n=85). Linear regression analysis showed a significant inverse relationship between the two parameters for the control group (R^2=0.322, $p<0.0001$, n=78), but not for the exposed group ($p>0.05$, n=84).