Chemosphere 257 (2020) 127259

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Acute and long-term effects of anionic polyacrylamide (APAM) on different developmental stages of two marine copepod species

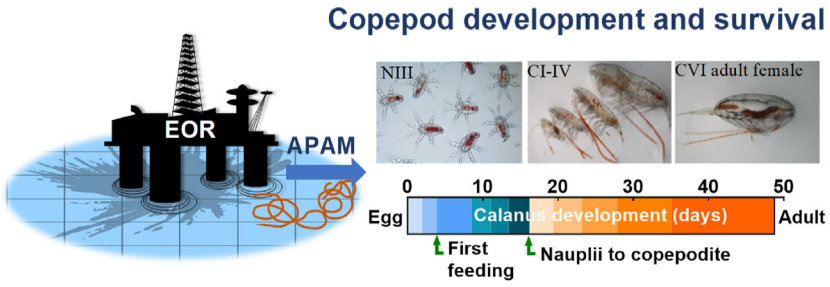
Julia Farkas [a, *], Dag Altin [b], Bjørn Henrik Hansen [a], Ida Beathe Øverjordet [a], Trond Nordtug [a]

[a] SINTEF Ocean, Environmental and New Resources, N-7465, Trondheim, Norway
[b] BioTrix, N-7022, Trondheim, Norway

HIGHLIGHTS

- No acute toxicity in adult life stages.
- Developmental effects in early life stages.
- Effects are related to the molecular size of the polymers.
- Effects are likely related to impacts on the energy budget.
- Effects limits are above anticipated sea water concentrations during offshore EOR.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 March 2020
Received in revised form
25 May 2020
Accepted 28 May 2020
Available online 5 June 2020

Handling Editor: Jim Lazorchak

Keywords:
Toxicity
Enhanced oil recovery
Polymers
APAM
Marine copepods
Early life stage

ABSTRACT

The application of synthetic polymers such as anionic polyacrylamides (APAM) in enhanced oil recovery (EOR) may increase in the future. This can lead to environmental release through offshore produced water discharges with so far limited knowledge on impacts in marine ecosystems. We investigated impacts of APAM polymers on two marine copepod species. Acute effects of APAM were studied on different life stages of *C. finmarchicus* (three molecular sizes: 200 kDa, 2800 kDa and 8000 kDa) and *Acartia tonsa* (one molecular size: 2800 kDa). Further, effects on development and survival following long-term exposure (spanning over several life stages) to 200 kDa APAM were studied in *C. finmarchicus*. Results show that none of the APAM molecules caused mortality in acute exposure experiments in adult *C. finmarchicus* even at high exposure concentrations ($\geq$1000 mg/L). Comparing toxicity of the 2800 kDa APAM between *C. finmarchicus* and the standard marine toxicity test copepod *Acartia tonsa* showed that the latter was slightly more sensitive. Early life stages of both copepods were more sensitive compared to later ones, and APAM exposure induced increased mortality and developmental delays. Effects were generally more pronounced for the larger polymers, most likely due to increased viscosity of the test dispersions leading to increased energy expenditures of the animals. However, significant effects were only observed at very high exposure concentrations that are probably higher than concentrations found in the environment.

1. Introduction

Polyacrylamides (PAM) are high molecular weight polymers deriving from the polymerization of acrylamide monomers. Their

* Corresponding author. SINTEF Ocean Environment and New Resources, Brattørkaia, 17C 7010, Trondheim, Norway.
E-mail address: julia.farkas@sintef.no (J. Farkas).

https://doi.org/10.1016/j.chemosphere.2020.127259

chemical composition and ionic character depend on the functional groups added to the acrylamide chemical moiety (Abidin et al., 2012; Acharya et al., 2010). They can occur in non-ionic form, or as polyelectrolytes in cationic (CPAM) and anionic (APAM) form and are produced in various molecular weights depending on their intended application. PAMs are widely used in industrial processes, amongst others to aid flocculation and complexation (Biesinger et al., 1976; Bolto and Gregory, 2007). Synthetic polymers, including APAM, are used by the oil industry to enhance recovery of oil (EOR) from geological formations through polymer flooding (PF) (Abidin et al., 2012; Standnes, 2014). In EOR processes polymers will follow the produced water fraction during oil production and can thereby enter the environment with produced water discharges. EOR polymers, including APAM, are generally assumed to be relatively nontoxic (Biesinger and Stokes, 1986; Bolto and Gregory, 2007). However, data on impacts on aquatic species, especially marine species are very limited. In a study exposing marine fish embryos and larvae to 200 kD APAM toxicity was observed only on very high concentrations (6000 mg/L) (Hansen et al., 2019). APAM is reported to be much less toxic to aquatic organisms compared to other synthetic polymers such as CPAM, which is probably why effects of APAM have been less studied (Beim and Beim, 1994; Biesinger et al., 1976; Hamilton et al., 1994). The use of different APAM types (size, charge density) further complicates the comparison between studies (Xiong et al., 2018). Findings on the influence of molecular size (chain length) on toxicity in aquatic organisms vary between studies. While Bolto and Gregory (2007) describe APAM with a long chain length as most toxic, other studies could not establish a direct correlation between chain length and toxicity (Beim and Beim, 1994; Hall and Mirenda, 1991).

So far, most studies investigated the effects of APAM on freshwater species as APAM is used in wastewater treatment processes and can be released to receiving freshwater bodies (Aguilar et al., 2002; Swift et al., 2015). Despite the potential exposure of marine species to APAM, e.g. through produced water discharges or mine tailing disposal, data on the potential effects on marine species such as copepods are scarce.

Calanus finmarchicus is the main filter feeding zooplankton species in the North Atlantic featuring a very high biomass and it plays a key role in the marine food chain by maintaining the flux of energy from microalgae to higher trophic levels (Sakshaug et al., 1994). *C. finmarchicus* share a common developmental scheme with most copepod species, with several naupliar and copepodite life stages that were seen to be more sensitive to stressors compared to adult individuals (Jager et al., 2016).

In this study we investigated acute and sublethal effects of APAM molecules of three molecular sizes on different life stages of *C. finmarchicus* to determine potential risks related to the release of APAM from offshore EOR. Experiments on early life stages spanned critical developmental transitions in the life history of the copepods like hatching, initiation of feeding and metamorphosis (from nauplii to copepodite). To provide better information for future risk assessments and interspecies comparison we further studied acute effect levels of one of the APAM molecules (2800 kDa) on *Acartia tonsa*, a copepod routinely used for standard toxicity testing. To our knowledge, this is the first study investigating the effects of APAM on different life stages of marine copepods.

2. Materials and methods

2.1. Preparation and characterization of APAM dispersions

The APAM samples were supplied in powder form by SNF (France). They had a low charge density, low to medium molecular size, and identical chemistry to APAM intended for industrial use. Three different molecular weights of the same polymer type were used in this study featuring average molecular weights of 200, 2800 and 8000 kDa, respectively. Stock dispersions of APAM were prepared according to standard procedures (SNF, 2007; QC-5001, ver. 3) by dissolving the dry powders in filtered (0.2 μm) natural seawater. The stock dispersions were subsequently filtered through a 100 μm nylon filter (Cell strainer, BD Falcon, USA) to remove eventual undispersed aggregates.

Viscosity measurements were used to indirectly determine concentrations of the prepared dispersions. Viscosity was measured at constant temperatures corresponding to that of the exposure experiments (10 °C for *C. finmarchicus* and 20 °C for *A. tonsa*) using a Brookfield viscometer LV-II + Pro (AMETEK Brookfield, Middleboro, MA, USA) with an UL adapter connected to a Brookfield TC-650 water bath as a cooling system. Results are shown in Fig. 1.

As APAM may attach to glassware, the experiments were conducted in standard or modified commercial polyethylene terephthalate bottles (PET bottles, Nordic Pack) and in 6 well cell-culture treated multi-dishes (Costar 3527, Corning Inc., NY USA).

2.2. Effect studies

2.2.1. Test organisms

Calanus finmarchicus features 12 developmental stages in addition to the egg stage: 6 naupliar stages (NI-NVI) and 6 copepodite (CI-CVI) stages. The transitions between all stages requires moulting. Morphological differentiation into females and males occur at the transition from copepodite stage V to VI (Marshall, 1954). We investigated the effects of APAM exposure on different life stages of *C. finmarchicus*, including three potentially vulnerable events in the life history, namely hatching, first feeding and the transition from nauplii to copepodites.

C. finmarchicus (Gunnerus) from a permanent laboratory culture were used in the experiments. The culture was initially established from stage V copepodites (CV) collected in Trondheimsfjorden, Norway. The culture is kept at 10 °C and is breeding continuously without diapause. Animals are maintained with a diet consisting of a mixture of three species of microalgae. Culturing conditions are described in detail in Hansen and co-workers (Hansen et al., 2007).

Acartia tonsa Dana is a calanoid copepod species with similar developmental features as *C. finmarchicus* as it has 12

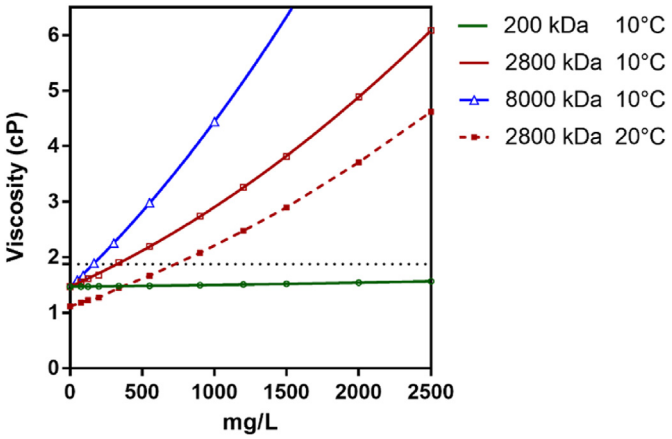


Fig. 1. Viscosity (cP) of 200, 2800 and 8000 kDa APAM in seawater at 10 °C, and 2800 kDa APAM at 20 °C. Dashed line indicate viscosity of seawater (SW) at 0 °C. Concentrations are given as nominal values based on the weight of dissolved APAM corrected for the water content of the solid polymer.

developmental stages in addition to the egg stage. Due to ease of culturing, and the fact that the eggs can be kept dormant in the refrigerator for months, makes the species widely used for standard toxicity testing.

Acartia tonsa were hatched from refrigerated in-house produced dormant eggs from the strain originally kept at the National Institute of Aquatic Resources, Charlottenlund, Denmark. The strain has been kept in the laboratory for several generations and is well adapted to the local conditions.

2.2.2. Acute effects of short-term APAM exposure

2.2.2.1. Acute toxicity on late copepod stages (CV-CVI). Acute toxicity tests on *C. finmarchicus* stage CV were performed as modified static copepod toxicity tests (ISO 14669:1999). The tests were conducted in closed 500 mL PET bottles containing 500 mL exposure dispersion and 10–15 mL headspace, with 10 individuals per vessel. The animals were exposed for 96 h at 10 °C in darkness with nominal exposure concentrations ranging from 280 to 10,000 mg/L for 200 kDa APAM, 75–2500 mg/L for 2800 kDa APAM and 28–1000 mg/L for 8000 kDa APAM (for further details see Supporting information Table S1). Differences in exposure concentrations between APAM molecules relate to their increasing viscosity with increasing molecular size. Mortality was assessed every 24 h, using immobility and opaque appearance as criteria for death. Each concentration was tested in triplicates ($n = 3$), with $n = 6$ for controls. As a positive control reference substance 3,5-dichlorophenol (0.8 mg/L; glass bottles) was used ($n = 3$) with a responsiveness of 20–80% set as validity criteria (ISO 14669:1999). At the start and at the end of the acute exposure experiment oxygen concentration and pH were recorded in one bottle from each exposure concentration and in two controls.

2.2.2.2. Effects on hatching and survival of early life stages (egg to NI/NII). Potential effects of APAM (200 kDa, 2800 kDa and 8000 kDa) on hatching time and hatching success of exposed eggs and the survival of early nauplii were tested. To obtain eggs, reproducing females from the culture were transferred into separate containers with clean seawater and provided with the unicellular algae *Rhodomonas baltica* (approximately 7500 cells/mL). Eggs were harvested after 16 h, resulting in an average age of the eggs of 8 h at the start of the 72 h exposure test. The eggs were distributed into 6 well multi-well plates (Costar 3527, Corning Inc., NY USA).) filled with 6 mL exposure dispersion or clean seawater as control. Exposure concentrations varied between the different molecule sizes and were between 105 and 3601 mg/L for 200 kDa, 56–2000 mg/L for 2800 kDa and 15–1000 mg/L for 8000 kDa APAM (Supporting information Table S2). Each exposure was performed in replicates ($n = 6$; controls: $n = 12$) with 31.7 ± 1 eggs on average in each replicate. The well plates were enclosed in plastic zip lock-bags to limit evaporation. Hatching time and success was determined every 24 h by counting the number of non-hatched eggs and eggs shells under a dissecting microscope. Survival was evaluated every 24 h by determining the number of motile versus dead (denatured/opaque and immobilized) nauplii and unhatched eggs. Average age at hatching was determined by fitting a cumulative distribution with fixed slope and calculating the 50% hatch relative to the final number of nauplii.

2.2.2.3. Acute effects on survival of the first feeding nauplii stage (NIII). Effects of APAM (200 kDa, 2800 kDa and 8000 kDa) on the survival of *C. finmarchicus* first feeding nauplii stage (NIII) was tested. Copepod eggs were incubated in 2 L glass bottles (Schott, Germany) at 10 °C until five days post hatch. The hatched nauplii were fed with the unicellular algae *R. baltica* (approximately 7500 cells/mL). When the nauplii reached stage NIII, active feeding was confirmed by observing ingested algae within the intestinal tract using a dissecting microscope. Feeding nauplii were transferred into 6 well multi-well dishes prefilled with exposure dispersions or clean seawater (control). Exposure concentrations are given in Supporting information (Table S3). Approximately 25 nauplii were added to each of the six replicates for each concentration together with *R. baltica* at an initial concentration of 9000 cells/mL. An additional batch of algae were added mid-way through the experiment. Two non-fed groups were added to the experimental setup, one group in clean seawater (control) and one at the highest exposure concentration. Mortality was recorded in all groups after 24 h, 48 h, and 72 h using immobility and opaque appearance (denaturation) as a criterion for death.

2.2.2.4. Acute effects on A. tonsa. To relate effects observed in *C. finmarchicus* to a standard copepod ecotoxicity test species, the acute toxicity tests on egg to NII, and adult life stages were performed in parallel on *A. tonsa* using the medium chain length APAM molecule (2800 kDa). Exposure concentrations are shown in Supporting information (Table S3). The test from egg to NII was performed like that described above for *C. finmarchicus* but at 20 °C. Eggs were collected from the culture over a period of 16 h. The acute toxicity test with adult *A. tonsa* was performed according to the ISO guideline (ISO14669:1999), with a test duration of 48 h and Nunc™ Cell Culture Flasks were used as exposure vessels.

2.2.3. Long-term effects of APAM exposure

Long-term effects of 200 kDa APAM on *C. finmarchicus* survival and development were tested on the first feeding nauplii stage (NIII) to copepodites stages CI/CII. To test the potential toxicity of APAM on copepods without confounding effects of viscosity of APAM, the experiments were performed with the 200 kDa polymer only. To obtain NIII, *C. finmarchicus* eggs were incubated in 2 L Schott glass bottles at 10 °C for hatching and development until stage NIII. Before reaching the NIII stage, algae (*R. baltica*) were supplied at approximately 7500 cells/mL until feeding stage NIII nauplii were observed. Actively feeding NIII, verified by observing algae in the gut, were then transferred to 330 mL PET bottles in a flow-through system and exposed to five concentrations of APAM, ranging from 625 to 10,000 mg/L (Supporting information, Table S4). APAM stocks were premixed and supplied with algae (*R. baltica*) at a concentration corresponding to 7500 cells/mL. The stocks were renewed every third day and kept in suspension by air bubbling. Exposure dispersions were continuously added to the lower part of the exposure bottles through a capillary at an average flow rate of 0.1 mL/min together with air (4 mL/min) to assure mixing.

Exposures were performed in quadruplicates ($n = 4$), with 8 control groups ($n = 8$). To monitor the development of the copepods, additional reference groups were kept in parallel. The experiment was terminated when approximately 75% of the nauplii in the reference groups had moulted into copepodites. Survival and developmental stage were determined in all exposure groups at the end of the exposure period. Criteria for mortality were identical to those described previously.

2.3. Statistics

Unless otherwise stated, statistics and fitted curves were prepared in GraphPad Prism version 6.01 or 7.0 (GraphPad, USA). Curve fits and calculation of LC_x-values were using sigmoid log dose-response plots, applying maximum and minimum restrictions for curves presenting normalized values (0 and 1). For the comparison of LC_{10} values, an extra sum of squares F-test was performed, testing if best fit values differ between datasets ($p < 0.05$). To

determine differences between exposure groups, data sets were analysed for normality (Shapiro-Wilk normality test) and analysed with one-way ANOVA followed by Tukey's multiple comparisons test.

3. Results and discussion

3.1. APAM dispersions

3.1.1. Viscosity of APAM dispersions in seawater

We determined the viscosity of the 200, 2800 and 8000 kDa APAM polymers used in experiments at different concentrations before the start of the exposure experiments. Our results show a clear correlation between viscosity, molecular size (APAM chain length) and concentration (Fig. 1). At 10 °C, the viscosity of APAM dispersions corresponded to seawater at 0 °C at concentrations of approximately 6700 mg/L (200 kDa), 370 mg/L (2800 kDa) and 120 mg/L (8000 kDa), respectively. During diapause, *C. finmarchicus* can remain in cold, dense water for several month and it is assumed that viscosities similar to that of seawater at 0 °C do not cause negative impacts (Visser and Jónasdóttir, 1999). The viscosity of 200 kDa APAM was tested up to the maximum exposure concentration of 10,000 mg/L, featuring a viscosity of 2.2 ± 0.03 (cP). This is slightly above the viscosity of seawater at 0 °C (1.89 cP) and10 °C (1.39 cP) (Ramsing and Gundersen, 1994).

3.2. Acute effects of short-term APAM exposure

3.2.1. Acute toxicity on late copepodite stages (CV-CVI)

No increased mortality was observed for any of the exposure groups with *C. finmarchicus* stage CV-CVI compared to control groups. Thus, the effect limits (LC_{10}) for mortality were above the highest concentrations tested, which were 10,000 mg/L for 200 kDa, 2500 mg/L for 2800 kDa, and 1000 mg/L for 8000 kDa. In positive control groups exposed to 3,5-dichlorophenol the mortality was 43.3%. *Acartia tonsa* was exposed to 2800 kDa APAM, and was, with a calculated 48 h LC_{10} concentration of 1184 mg/L (714–1962 mg/L), slightly more sensitive than *C. finmarchicus* (48 h LC_{10} > 2500 mg/L). Our results show that the tested APAM is not acutely toxic to *C. finmarchicus* late copepodite stages (CV and CVI) and causes effects in *A. tonsa* only at very high, not environmentally relevant exposure concentrations (<199 mg/L) (Hansen et al., 2019). To our knowledge, no studies investigating the effects of pure APAM on marine copepods have previously been published. However, results are in agreement with a risk assessment study testing acute toxicity of mine tailings in combination with the polymer flocculants polyDADMAC (Magnafloc® LT38, high cationic charge density) and APAM (Magnafloc®10, low charge density, high molecular weight), which reported no acute effects on *A. tonsa* at concentrations relevant for mine tailing release (up to 99 mg/kg APAM) (Berge et al., 2012). Beim and Beim (1994) studied the impacts of different polymers, including APAM and CPAM on several freshwater species. They report a large deviation in acute effects of the tested APAM (Magnafloc®10) between the two tested freshwater crustaceans, with 96 h LC_{50} values of 14.1 mg/L for *Daphnia magna* and >2100 mg/L for the large gammarid *Eulimnogammarus verrucosus*.

3.2.2. Effects on hatching and survival of early life stages (egg to NI/NII)

Hatching time, hatching success and nauplii survival was determined in very early life stages (eggs to NI/NII) of copepods following APAM exposure (200 kDa, 2800 kDa and 8000 kDa, respectively). The average time from shedding of eggs to 50% hatching for all *C. finmarchicus* groups ($n = 163$) was 29.4 ± 1.1 h (Fig. 2A − C), with no significant differences between the control groups and exposed groups (Fig. 2A). Hatching success in eggs exposed to 200 kDa APAM was significantly ($p = 0.0001$) reduced at the two highest exposure concentrations (1979 mg/L and 3601 mg/L) (Fig. 2A). No effects on hatching success and time of hatching were observed for *C. finmarchicus* during exposure to the other two APAM sizes, with the highest tested concentrations of 2000 mg/L for 2800 kDa APAM and 1000 mg/L for 8000 kDa (Fig. 2B and C).

Similarly, no reduction in hatching success or time of hatching were observed in *A. tonsa* eggs exposed to 2800 kDa APAM in the range of 56–2000 mg/L (Fig. 2D). The estimated average age of the eggs at 50% hatching for *A. tonsa* was 27.4 ± 0.4 h ($n = 54$).

In contrast to hatching success, mortality in hatched *C. finmarchicus* nauplii (NI/NII) increased significantly and in a concentration-dependent manner for all APAM exposures (Fig. 3). This shows that early nauplii were more sensitive to APAM compared to eggs and adult individuals. Nauplii mortality in exposures was significantly higher than in controls at concentrations ≥1097 mg/L for 200 kDa ($p = 0.0006$), 1100 mg/L for 2800 kDa ($p = 0.0007$) and 550 mg/L for 8000 kDa ($p = 0.0006$) exposures (Fig. 3A, B, C). Corresponding LC_{10} concentrations were 892 mg/L, 714 mg/L and 417 mg/L for 200 kDa, 2800 kDa and 8000 kDa APAM, respectively. This shows that the effect concentrations for early nauplii mortality were inversely correlated with molecule size. This is in agreement with Bolto and Gregory (2007), who state that toxicity of APAM increases with molecular chain length (Bolto and Gregory, 2007). In contrast, other studies did not find a relation between molecular chain length and toxicity, but report that charge density (CD) influenced toxicity (Beim and Beim, 1994; Hall and Mirenda, 1991). However, CD of the different APAM sizes used in our study were similar (SNF Floeger, personal communication). All polymers studied here were well above the molecular size limit for passive diffusion into cells, which is about 1 kDa (Matsson and Kihlberg, 2017). It is thus likely that external physical changes caused the observed effects. Increased viscosity, causing increased energy demands for organisms in motion, can play a role for the observed effects, especially for the larger chain-length molecules. However, it is not likely the sole reason for the effects, as mortality also occurred in the 200 kDa exposure at concentrations which were below those causing a viscosity (1.5 cP at 1200 mg/L) increase to above the normal range for seawater at relevant temperatures (viscosity seawater: 1.87 at 0 °C, 1.39 at 100 °C). Mechanical action through attachment to organism surfaces, blocking vital body functions such as feeding and digestion, was previously described as the main "mode of action" of PAMs (Beim and Beim, 1994). This could result in increased stress for smaller organisms and especially for early life stages with limited energy reserves. While feeding and digestion are not yet relevant for nauplii up to stage NII (*A. tonsa*) or NIII (*C. finmarchicus*), other impacts such as reduced respiration through decreased O_2 diffusion could play a role. This would be even more pronounced at higher temperatures, i.e. 20 °C in the *A. tonsa* exposure experiments. Comparing the two copepod species, we observed that *A. tonsa* nauplii were slightly more sensitive than *C. finmarchicus*, with significant impacts on survival occurring at ≥330 mg/L ($p < 0.05$). LC_{10} values for *A. tonsa* were, with 559 mg/L slightly, but not significantly ($p = 0.2502$) lower than for *C. finmarchicus*, with an LC_{10} of 714 mg/L (Fig. 3 D, Table 1). This is in agreement with the acute toxicity studies on adult copepods.

3.2.3. Acute effects on survival of first feeding nauplii (NIII)

Toxicity tests on the first feeding stage of *C. finmarchicus* (NIII) showed that 2800 and 8000 kDa APAM caused increased mortality compared to control animals at high exposure concentrations (Fig. 4). While we observed a reduced uptake of food into the guts in

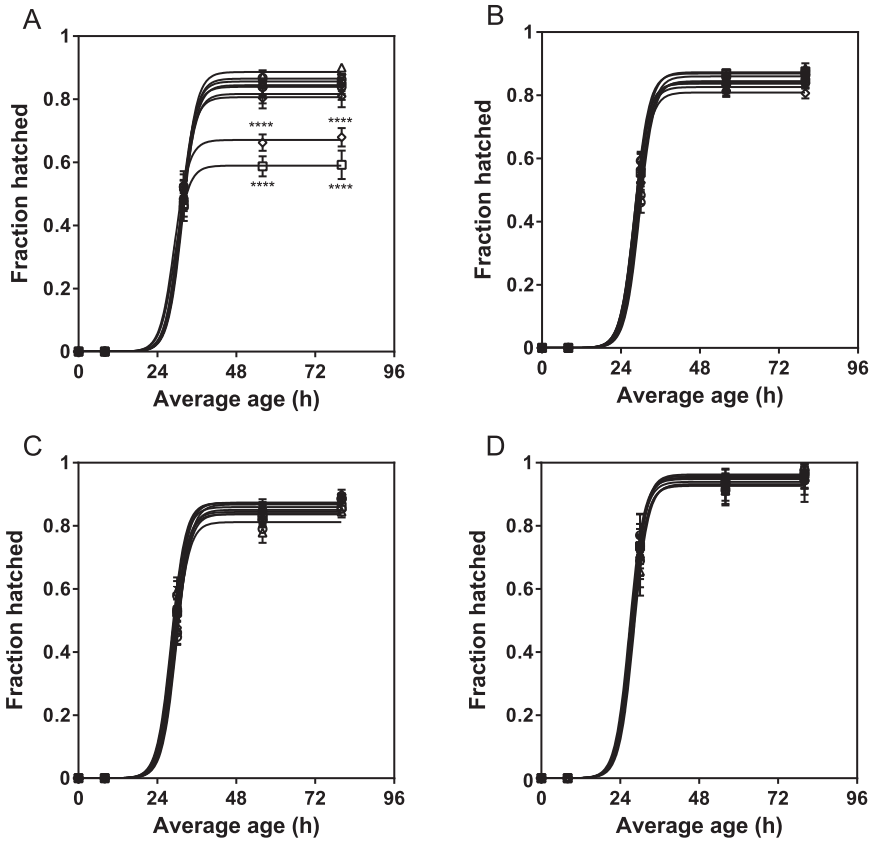


Fig. 2. Fraction of the initial *C. finmarchicus* egg population that hatched during the 72 h exposure to APAM with a chain length of 200 kDa (A), 2800 kDa (B) and 8000 kDa (C). Hatching time and success in *A. tonsa* exposed to 2800 kDa APAM is shown in panel D. The fitted curves (with respective symbols) represent the different exposure concentrations used for each APAM chain length (for details see Supporting information Table S2). The data is presented as average ± SE. Note the differences in maximum exposure concentrations, which were 3601 mg/L for 200 kDa APAM (A); 2000 mg/L for 2800 kDa (B and D) and 1000 mg/L for 8000 kDa (C). Fitted curves have been restricted to a fixed slope that is identical for all groups. Asterisks in Figure A denotes significant differences in hatching success ($p < 0.0001$).

high exposure concentrations of the large polymers (data not shown), the presence or absence of food did not directly affect the survival of copepods in the current experiments, as the starved and fed groups had largely identical survival in both controls and the highest exposure concentrations (Fig. 4). Different effect levels were observed for the three tested polymer size classes. Similar to survival in early nauplii (Fig. 3), mortality occurred at lower concentrations for the larger polymers, with almost no impacts seen for 200 kDa APAM below 10,000 mg/L. The lowest LC_{10} concentration was observed for 8000 kDa APAM (144 mg/L). However, effects of 2800 kDa APAM were more severe at higher concentrations (Fig. 4). The dose-response curve for the 8000 kDa polymer (Fig. 4C) further deviates from a common s-shaped dose-log response curve, being almost linear over a large range of exposure concentrations. This is potentially related to decreased activity levels (swimming activity) of nauplii in 8000 kDa compared to the other APAM exposures (observation, data not shown). NIII nauplii, which are dependent on external energy through active feeding by filtration, potentially spend more energy moving actively in a high viscosity medium than they can compensate for by feeding. In more viscous media, reduced activity can help saving energy expenditures. A similar phenomenon, namely the decrease of activity levels in high exposure scenarios leading to increased survival in copepod early life stages exposed to mine tailings was recently described (Farkas et al., submitted). This could also explain that there were only minor differences between the mortality of the starved groups and the corresponding fed groups.

Our results indicate that impairment of feeding, as reported in previous studies, probably contributes, but is not the sole cause of the observed mortality (Acharya et al., 2010, Beim and Beim, 1994), and that impacts are rather related to the overall energy balance, an interplay of energy availability, uptake and expenditures.

The impact of different APAM exposure concentrations and molecular sizes on feeding and energetics in copepods, however, remains elusive and should be subjected to further studies.

3.3. Long-term effects of 200 kDa APAM exposure

3.3.1. Effects on survival and development from first feeding nauplii stage (NIII) to copepodite stage CI/CII

The effects of a 14 d exposure to 200 kDa APAM on survival and development of *C. finmarchicus* early life stages, starting with first feeding nauplii stage (NIII), are shown in Fig. 5. In contrast to short-term exposure (72 h; Fig. 4), long-term exposure to high concentrations of 200 kDa APAM led to decreased survival of early life stage copepods. Survival (sum all stages) was significantly reduced at 2500 mg/L ($p = 0.0002$) (Supporting information Figure S2). At 5000 mg/L almost all individuals died, and exposure to 10,000 mg/L resulted in a 100% mortality (Fig. 5A). Further, the stage distribution of *C. finmarchicus* at the termination of the experiment changed in a concentration dependent manner, with less animals developing into CI and CII copepodites at higher concentrations (Fig. 5A). The relative abundance of copepodites versus nauplii at the termination of the experiment was used to estimate the effect of the exposure on the rate of development. Fig. 5B shows that the fraction of copepodites in the surviving population decreased in a

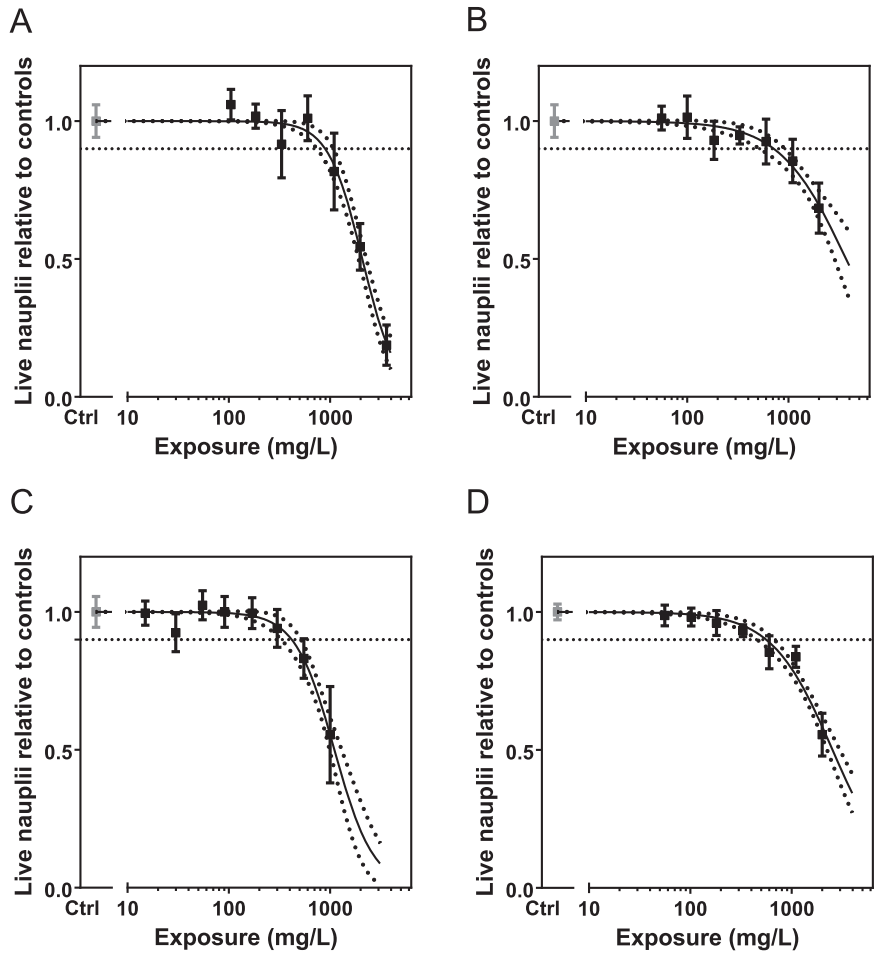


Fig. 3. Fraction of live *C. finmarchicus* nauplii (NI/NII) compared to controls after 72 h exposure to 200 kDa (A), 2800 kDa (B) and 8000 kDa (C) APAM at 10 °C. Effects on *A. tonsa* nauplii survival after 72 h exposure to 2800 kDa APAM at 20 °C are shown in panel D. Data are presented as mean ± SD, and dotted lines indicating the 95% confidence interval. Number of replicates: 12 for controls and 6 for each exposure concentration. Straight horizontal dotted line indicates 10% increase in mortality compared to controls.

Table 1
Summary of effect limits expressed as nominal LC/EC10 for different tests with three different molecular sizes of APAM. Numbers in brackets indicate 95% confidence interval (min − max).

						LC_{10}/EC_{10} (min-max) mg/L		
						APAM molecular size		
Test	Endpoint	Species	Stage	Dura-tion	°C	200 kDa	2800 kDa	8000 kDa
Acute toxicity	Survival	*C. finmarchicus*	CV/CVI	96 h	10 °C	>10,000	>2500	>1000
Acute toxicity	Survival	*A. tonsa*	CV	48 h	20 °C		1184 (714−1962)	
Early life stage test	Survival	*C. finmarchicus*	Egg-NI/NII	72 h	10 °C	892 (734−1084)	714 (534−954)	417 (330−528)
Early life stage test	Survival	*A. tonsa*	Egg-NI/NII	72 h	20 °C		559 (459−680)	
Early life stage test	Survival	*C. finmarchicus*	NIII	72 h	10 °C	>10,000	461 (432−492)	144 (118−177)
Long-term exposure	Survival	*C. finmarchicus*	NIII−CI/CII	14 d	10 °C	1664 (905−3058)		
Long-term exposure	Develop-ment	*C. finmarchicus*	NIII−CI/CII	14 d	10 °C	517 (358−757)		

concentration-dependent manner over the concentration range tested (625−5000 mg/L), displaying a negative relationship between the rate of development and APAM exposure concentration. The concentration corresponding to a 10% reduction (EC_{10}), in copepodite fraction relative to the control after 14 days of exposure was estimated to be 517 mg/L APAM (range 358−757; Supporting information Figure S1).

Thus, the results indicate that 200 kDa APAM can delay the development of the copepods at high exposure concentrations (≥625 mg/L). The duration of the delay in development can be roughly estimated from the duration of the CI stage, which is approximately 3.3 days at the temperature recorded during the experiment (9 °C). At 2500 mg/L, the fraction of CI is approximately the same as the fraction of CII in the control. Assuming the age

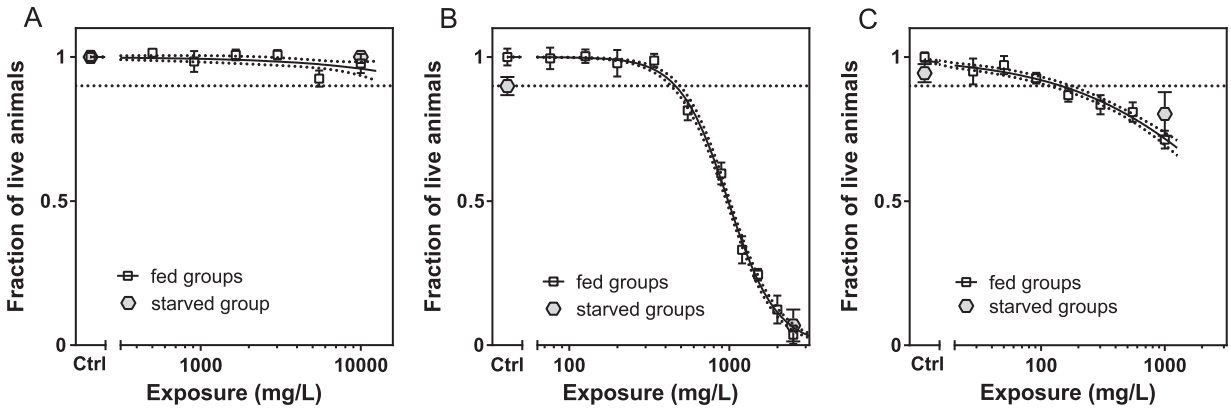


Fig. 4. Survival of *C. finmarchicus* nauplii NIII after 72 h exposure to (A) 200 kDa, (B) 2800 kDa, and (C) 8000 kDa APAM. Open squares indicate groups fed with *R. baltica* (9000 cells/mL), open hexagons represent starved reference groups. Data are presented as mean ± SD with dotted lines indicating the 95% confidence interval. Vertical dotted lines indicate concentrations where viscosity corresponds to seawater at 0 °C. Number of replicates per concentration are 6 for the exposure groups and 12 for the controls. Horizontal straight dotted lines indicate a 10% increase in mortality compared to controls. Note the different scaling on the X-axes. All values including starved groups are plotted relative to the average of the fed control groups.

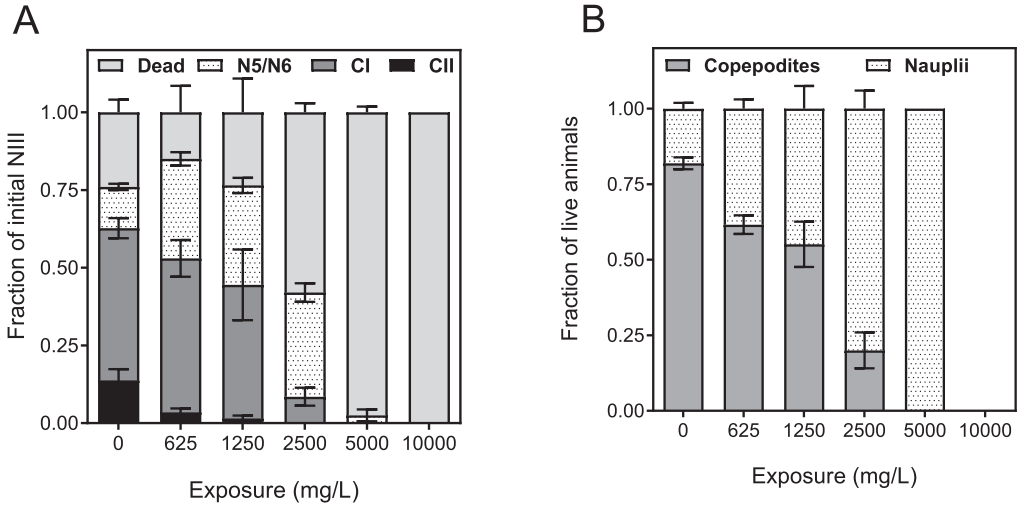


Fig. 5. Stage distribution and survival of *C. finmarchicus* after 14 days exposure to 200 kDa APAM from the first feeding stage (NIII) to copepodite stages CI/CII. (A) Stage distribution of surviving copepods and fraction of dead copepods after the 14 d exposure relative to the original number of NIII nauplii showing the fraction of individuals in CII stage (black), CI stage (dark grey), N5/N6 (dotted) and dead individuals (light grey). (B) Fractions of copepods and nauplii in the various exposure groups All data are presented as average ± SE. Number of replicates: 12 for controls and 6 for the exposure groups.

distribution to follow a Hill-curve as described by Cook et al. (2007), the delay would be approximately 3.3 days for the 2500 mg/L exposure, which corresponds to a 23% increase in developmental time from NIII to CI (Cook et al., 2007). Accordingly, the delay in development is expected to be considerably less than 3 days below 2500 mg/L exposure (Fig. 5 A, Supporting informationFigure S1). For comparison, Cook et al. (2007) observed a delay of 22% in development for the late nauplii stages NIII to NVI of *C. finmarchicus* when exposed to low carbon diets compared to high carbon diets (Cook et al., 2007). This may suggest that the delay in the current experiment is also related to energetic conditions caused by reduced energy intake (feeding) and/or increased energy consumption (activity). This supports our findings on reduced survival in the first feeding stages (NIII) following short-term exposure to the larger and more viscous 2800 and 8000 kDa APAM (Fig. 4). The observed effects can thus likely be related to impacts on the animals' energy budget, as described previously for *D. magna* (Acharya et al., 2010).

3.4. Environmental toxicity

While early life stages were more sensitive to APAM exposure than eggs and adults, effects generally occurred at very high exposure concentrations with elevated viscosity of the test dispersions. Long-term exposure caused an estimated 10% delay in development for early life stages at 517 mg/L for the smallest polymer (200 kDa). The lowest observed LC_{10} of 144 mg/L was observed for survival of *C. finmarchicus* first feeding stage exposed to 8000 kDa polymer (Table 1).

Reports on concentrations of polymer are rare, but reports from the Shengli Oilfield (Dongying, China) showed about 170 mg/L polymer in the produced water (Zhang, 2010). This is slightly above the lowest effect limit (EC_{10}) observed in the current work (Table 1). Produced water is rapidly diluted in the sea and realistic exposure concentrations a few metres from the release point (Hansen et al., 2019) are therefore expected to be significantly below the observed effect limits for *C. finmarchicus*. However, degradation rates of polymers in the environment are low (Guezennec et al., 2015), and more work is needed to determine real environmental

concentrations for evaluating potential impacts. Further, more research is needed to better understand effect mechanisms, and thus the causes for different effect limits between test species and different life stages.

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

Julia Farkas: Formal analysis, Writing - original draft, Writing - review & editing. **Dag Altin:** Conceptualization, Data curation, Investigation, Methodology, Validation, Visualization, Writing - review & editing. **Bjørn Henrik Hansen:** Conceptualization, Data curation, Writing - review & editing. **Ida Beathe Øverjordet:** Formal analysis. **Trond Nordtug:** Conceptualization, Data curation, Methodology, Validation, Project administration, Resources, Supervision, Visualization, Writing - review & editing.

Acknowledgments and funding

The work was financed by Equinor AS (contract number 4503331201). The polymers used for exposure were supplied by SNF. Thanks to Equinor AS (B. Gjersvold) for performing viscosity measurements of exposure dispersions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.127259.

References

Abidin, A.Z., Puspasari, T., Nugroho, W.A., 2012. Polymers for enhanced oil recovery technology. Procedia Chemistry 4 (Suppl. C), 11–16.
Acharya, K., Schulman, C., Young, M.H., 2010. Physiological response of Daphnia magna to linear anionic polyacrylamide: ecological implications for receiving waters. Water Air Soil Pollut. 212 (1–4), 309–317.
Aguilar, M.I., Sáez, J., Lloréns, M., Soler, A., Ortuño, J.F., 2002. Nutrient removal and sludge production in the coagulation-flocculation process. Water Res. 36, 2910–2919.
Beim, A.A., Beim, A.M., 1994. Comparative ecological-toxicological data on determination of maximum permissible concentrations (mpc) for several flocculants. Environ. Technol. 15 (2), 195–198.
Berge, J., Beylich, B., Brooks, S., Jaccard, P., Tobiesen, A., Øxnevad, S., 2012. Overvåking Av Bøkfjorden 2011 Og Giftighetstesting Av Gruvekjemikaliene Magnafloc LT 38 Og Magnafloc 10. NIVA report).
Biesinger, K.E., Lemke, A.E., Smith, W.E., Tyo, R.M., 1976. Comparative toxicity of polyelectrolytes to selected aquatic animals. J. Water Pollut. Control Fed. 48 (1), 183–187.
Biesinger, K.E., Stokes, G.N., 1986. Effects of synthetic polyelectrolytes on selected aquatic organisms. Journal (Water Pollution Control Federation) 58 (3), 207–213.
Bolto, B., Gregory, J., 2007. Organic polyelectrolytes in water treatment. Water Res. 41 (11), 2301–2324.
Cook, K.B., Bunker, A., Hay, S., Hirst, A.G., Speirs, D.C., 2007. Naupliar development times and survival of the copepods Calanus helgolandicus and Calanus finmarchicus in relation to food and temperature. J. Plankton Res. 29 (9), 757–767.
Farkas, J., Svendheim, L., Hansen, B.H., Altin, D., Nordtug, T., Olsvik, P. A., Jager, T., Øverjordet, I. Effects of Mine Tailing Exposure on the Development of Early Life Stages of the Marine Copepod Calanus finmarchicus. (Submitted).
Guezennec, A.G., Michel, C., Bru, K., Touze, S., Desroche, N., Mnif, I., Motelica-Heino, M., 2015. Transfer and degradation of polyacrylamide-based flocculants in hydrosystems: a review. Environ. Sci. Pollut. Control Ser. 22, 6390–6406.
Hall, W.S., Mirenda, R.J., 1991. Acute toxicity of waste-water treatment polymers to daphnia-pulex and the fathead minnow (Pimephales-Promelas) and the effects of humic-acid on polymer toxicity. Res. J. Water Pollut. Contr. Fed. 63 (6), 895–899.
Hamilton, J.D., Reinert, K.H., Freeman, M.B., 1994. Aquatic risk assessment OF polymers. Environ. Sci. Technol. 28 (4), 186A–192A.
Hansen, B.H., Malzahn, A., Hagemann, A., Farkas, J., Skancke, J., Altin, D., Nordtug, T., 2019. Acute and sub-lethal effects of an anionic polyacrylamide on sensitive early life stages of Atlantic cod (Gadus morhua). Sci. Total Environ. 652, 1062–1070.
Hansen, B.H., Altin, D., Nordtug, T., Olsen, A.J., 2007. Suppression subtractive hybridization library prepared from the copepod Calanus finmarchicus exposed to a sublethal mixture of environmental stressors. Comp. Biochem. Physiol. Genom. Proteonomics 2, 250–256.
Jager, T., Altin, D., Miljeteig, C., Hansen, B.H., 2016. Stage-dependent and sex-dependent sensitivity to water-soluble fractions of fresh and weathered oil in the marine copepod *Calanus finmarchicus*. Environ. Toxicol. Chem. 35 (3), 728–735.
Marshall, S.M.a.O., A P, 1954. Hatching in Calanus finmarchicus and some other copepods. J. Mar. Biol. Assoc. U. K. 33 (2), 393–401.
Matsson, P., Kihlberg, J., 2017. How big is too big for cell permeability? J. Med. Chem. 60 (5), 1662–1664.
Ramsing, N., Gundersen, J., 1994. Seawater and Gases. Tabulated Physical Parameters of Interest to People Working with Microsensors in Marine Systems. Version 1.0. Revised Tables Compiled at. Max Planck Institute for Marine Microbiology, Bremen, Germany.
Sakshaug, E., Bjorge, A., Gulliksen, B., Loeng, H., Mehlum, F., 1994. Structure, biomass distribution, and energetics of the pelagic ecosystem in the barents sea - a synopsis. Polar Biol. 14 (6), 405–411.
Standnes, D.C., Skjevrak, I., 2014. Literature review of implemented polymer field projects. J. Petrol. Sci. Eng. 122, 761–775.
Swift, T., Swanson, L., Bretherick, A., Rimmer, A., 2015. Measuring poly(acrylamide) flocculants in fresh water using inter-polymer complex formation. Environ. Sci. J. Integr. Environ. Res.: Water Research and Technology 1 (3), 332–340.
Visser, A.W., Jónasdóttir, S.H., 1999. Lipids, buoyancy and the seasonal vertical migration of Calanus finmarchicus. Fish. Oceanogr. 8 (s1), 100–106.
Xiong, B.Y., Loss, R.D., Shields, D., Pawlik, T., Hochreiter, R., Zydney, A.L., Kumar, M., 2018. Polyacrylamide degradation and its implications in environmental systems. Npj Clean Water 1.
Zhang, Y., Gao, B., Lu, L., Yue, Q., Wang, Q., Yuyan Jia, Y., 2010. Treatment of produced water from polymer flooding in oil production by the combined method of hydrolysis acidification-dynamic membrane bioreactor–coagulation process. J. Petrol. Sci. Eng. 74, 14–19.