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Experimental studies of reproduction and feeding for two Arctic-dwelling *Calanus* species exposed to crude oil

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ABSTRACT: Copepods of the genus *Calanus* are keystone species in the transfer of energy from lower to higher trophic levels of the Arctic/sub-Arctic food web. We performed experimental tests on the reproduction and feeding of *Calanus* spp. exposed to the water-soluble fraction (WSF) of crude oil. Fecal pellet and egg production were examined for females of *C. glacialis* exposed to WSF (16 EPA) concentrations of 10.4 µg l^{-1} (high treatment; HT), 3.6 µg l^{-1} (low treatment; LT) and 0 µg l^{-1} (control treatment; CT). We observed no significant difference in cumulative egg or fecal pellet production. Egg hatching success was examined for 2 d after transferring eggs from treatment solutions to uncontaminated seawater. Hatching success was significantly lower in the HT compared to the CT. In a second experiment, feeding of *C. finmarchicus* was examined after exposure for 11 and 18 d to 7.0 (HT), 3.4 (LT) or 0 (CT) µg l^{-1} of WSF (16-EPA). Using algae cell concentrations as a proxy for feeding success, feeding was inhibited for *C. finmarchicus* specimens exposed to the HT of WSF compared to the CT. Our findings indicate that adult females of *C. glacialis* may withstand some exposure to crude oil components but the survival of offspring is negatively affected. Reduced feeding efficiency in *C. finmarchicus* exposed to high concentrations of WSF provides evidence that adult specimens are sensitive to exposure to crude oil. The study expands on the limited body of knowledge of potential changes to key life history traits of Arctic *Calanus* species resulting from exposure to chemical compounds in crude oil.

KEY WORDS: Arctic · *Calanus* species · Crude oil · Exposure · Life history traits

INTRODUCTION

Increasing shipping of petroleum products is underway in association with expanding petroleum exploration and development activities in Arctic waters. In the Barents Sea and along the coast of northern Norway the yearly transport of oil is projected to reach about 100 million tons within a decade (Bambulyak & Frantzen 2009). As a result, there is an increased risk of accidents with potential consequences for valuable marine resources and ecosystems as a whole. The impact of an oil spill in the marine environment is dependent on many factors including oil composition, time of year, spill location and clean-up actions (Fingas & Hollebone 2003, National Research Council 2003). Conditions in the Arctic, e.g. remoteness, a high frequency of unfavorable weather conditions and the presence of winter sea ice in some areas, poses special challenges for oil spill response and clean-up. Knowledge of the effects of crude oil exposure on key Arctic organisms will improve predictions of the outcome of oil spill events in cold-water ecosystems and facilitate the development of more effective contingency plans.

There is currently only limited information on the effects of petroleum compounds on cold-water adap-

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ted organisms and the use of toxicity data derived from studies of temperate species as predictors of effects on Arctic species has been called into question (Olsen et al. 2007a,b, 2008, Camus & Olsen 2008). In general, Arctic species life spans are longer than more southern sibling species (Maclean 1973, Koszteyn et al. 1995, Hirche 1997) which could lead to longer contaminant exposure times and/or higher body burdens over the lifetime of an organism. Furthermore, Arctic organisms contain high lipid concentrations which allow them to survive for long periods of starvation during winter (Lee 1974, Falk-Petersen et al. 1990) but also to efficiently store lipophilic contaminants (Lassiter & Hallam 1990, de Maagd et al. 1997).

Copepod species of the genus *Calanus* are, due to their size and effective synthesis of lipids (Scott et al. 2002), important in the transfer of energy from lower to higher trophic levels in the Barents Sea food web (Loeng & Drinkwater 2007). Several Barents Sea commercial fish species (e.g. capelin and herring) depend on *Calanus* species as a food resource (Loeng & Drinkwater 2007). In the Barents Sea 3 herbivore species of the genus *Calanus* occupy a similar niche in the food web but differ in their temperature and depth preferences (Daase et al. 2007). Atlantic water masses contain the smaller and leaner *C. finmarchicus* (copepodite 5 [CV] prosome length ≤ 2.7 mm; Arnkvaern et al. 2005). This species has a 1-yr life cycle at its northern limit of appearance (Arnkvaern et al. 2005). The distribution of *C. finmarchicus* overlaps with that of the true Arctic shallow water species, *C. glacialis*, and the latter dominates as we move into Arctic waters. *C. glacialis* is larger (CV prosome length >2.7 to 4.1 mm; Arnkvaern et al. 2005), has a higher lipid content and a 1 to 2-yr life cycle (Scott et al. 2000). *C. hyperboreus* is the largest of the 3 species (CV prosome length > 4.1 mm; Arnkvaern et al. 2005) and is found in Arctic and/or deep water areas (Hirche 1997).

While mortality currently serves as the basis for regulatory decision-making, concern is shifting toward non-lethal effects of oil exposure on species life history traits. Changes in feeding, behavior, egg production and survival of offspring after exposure will play a role in the long–term survivability of a species population (Chapman & Riddle 2005). Furthermore, these effects may be assessed earlier and at lower concentrations of oil exposure. Of all chemical components found in crude oil, polycyclic aromatic hydrocarbons (PAHs) are considered the most toxic (Hylland 2006); thus most crude oil effect studies focus on PAHs. In general, invertebrates, and presumably copepods, have a relatively low and variable ability to metabolize PAHs (Varanasi 1989, Rust et al. 2004) and thus may accumulate PAHs (Duesterloh et al. 2002, Rust et al. 2004, Carls et al. 2006). Experimental investigations have shown reduced feeding in *Calanus helgolandicus* exposed to the water soluble fraction (WSF) of Kuwait crude oil (Spooner & Corkett 1979). Cowles & Remillard (1983) observed reduced feeding but no change in egg production in *Centropages hamatus* exposed to >10 ng l^{-1} of crude oil while both egg production and egg hatching success was reduced in *Acartia tonsa* exposed singly to fluoranthene, phenanthrene and pyrene (Bellas & Thor 2007). The toxic effects of some PAHs are additive in *Oithona davisae* (Barata et al. 2005), which may also apply to other species.

To our knowledge, only 3 studies have been published on the effect of crude oil constituents on sub-Arctic and Arctic copepod species. Duesterloh et al. (2002) found the toxic effect of crude oil on sub-Arctic copepod species to be enhanced by UV-radiation. Hjorth & Dahllöf (2008) found increased mortality and decreased food intake and growth with increasing concentrations of pyrene in a sub-Arctic population of *Microsetella* sp. Life history traits on *Calanus finmarchicus* and *C. glacialis* exposed to pyrene were examined in a study from Disko Bay, Greenland (Jensen et al. 2008). Jensen et al. (2008) performed parallel experiments to examine egg production and feeding (using fecal pellet production as a proxy of feeding) on the 2 sibling species, and concluded that *C. finmarchicus* is more severely affected by exposure to pyrene than *C. glacialis*. Moreover, they argue that egg production was lowered as a consequence of reduced feeding in exposed copepods (Jensen et al. 2008).

Here we examine changes in life history traits for 2 of the 3 *Calanus* species present in the Barents Sea (i.e. *C. glacialis* and *C. finmarchicus*). We report on 2 different experiments, one examining feeding (as fecal pellet production), egg production and hatching success of *C. glacialis* and one examining only feeding (as algae cell reduction and fecal pellet production) in *C. finmarchicus* exposed to sub-lethal concentrations of oil (WSF). Exposure to the WSF allows us to include the additive toxic effects associated with a complex mixture of PAHs such as would be expected in an oil spill event (Barata et al. 2005). These studies of life history traits for a keystone species of the Arctic/sub-Arctic food web further aid in the evaluation of exposure limits for individual organisms and for evaluating possible population level effects resulting from exposure to chemical compounds in crude oil.

MATERIALS AND METHODS

The experiment on *Calanus glacialis* was performed over 12 d (12–23 May 2008) on adult females maintained in individual beakers. Each female was continuously exposed to a mixture of crude oil WSF and algae.

Eggs and fecal pellets were collected daily from each female. The collected eggs were maintained in uncontaminated sea water to monitor hatching success. The experiment on *C. finmarchicus* was performed over 22 d (26 June–17 July 2008) on CV copepodites, the dominant stage of this species found in the Barents Sea in June–July (Melle & Skjoldal 1998). Using a flow-through system, copepods were continuously exposed to crude oil WSF with no food additions. Feeding success was subsequently examined by incubating single females in an algae suspension for 24 or 72 h.

Egg and feeding experiment on *Calanus glacialis*. Experimental copepods were collected in Billefjorden, Svalbard (78° 40′ N, 16° 40′ E) on April 28, 2008. As the area was ice covered, a WP-2 net (180 µm mesh size) was operated through a hole in the ice. The water column (180 m) was stable with water temperature around −1.8°C and salinity 34.5. A vertical haul was taken from the bottom to the surface and the content of the cod-end was carefully transferred to thermo bottles for transport to the University Centre of Svalbard (UNIS). The copepods were kept in 50 l polypropylene containers in a dark climate room at 5°C for 6 d before being transferred by plane to Tromsø, Norway. Copepods were kept under similar laboratory conditions in the experimental facility at the University of Tromsø.

Seawater used for the experiments was natural seawater filtered through 20 and 5 µm cartridge filters. Three different concentrations of North Sea crude oil WSF were prepared by pumping filtered sea water through columns containing oil-covered glass beads (Duesterloh et al. 2002, Camus & Olsen 2008). The amount of oil used was 0, 1.75 and 3.5 g oil per kg glass beads. The oil/glass bead mixtures were dried for one week in a hood and later stored in sealed columns until the start of the experiment. Each column contained 1.5 kg glass beads.

Using a peristaltic pump (Watson Marlow 205U/CA) mounted with marprene tubing, water was passed through each column at a flow rate of 19.3 ml min^{-1}. Each day, 2 l of each concentration were produced for mixing with algae suspensions (*Thalassiosira* sp.) to a final concentration of 4000 cells ml^{-1}, a concentration previously shown to maintain egg production in the sibling species *C. finmarchicus* (Jensen et al. 2006). The algae were from a culture kept at 5°C in exponential growth phase on f/2 medium (Guillard & Ryther 1962) with silica addition. The experiment was run in 150 ml polypropylene beakers containing 100 ml water, with the individual copepods kept in an inner beaker with a false bottom of plankton netting (300 µm). The day before the experiment began single females were held in individual beakers containing clean filtered sea water. The experiment was started by transferring individuals to randomly selected outer beakers containing the water/algal mixture for one of the 3 treatments (control treatment: CT; low treatment: LT; high treatment: HT). A total of 20 replicates was run for each treatment. Each day, females were transferred to a new pre-filled outer beaker containing the same treatment mixture. During the transfer, the condition of females was evaluated and dead animals were removed (2 in CT, 3 in LT and 3 in HT; these were excluded from analyses). Eggs and fecal pellets were obtained by filtering the remaining water from the used beakers through plankton netting (80 µm). Eggs and fecal pellets were counted daily. Throughout the 11 d experiment, water temperature was maintained at 3°C and dimmed light was provided 24 h to resemble the natural light cycle of the Arctic summer.

The size of fecal pellets was estimated by measuring 20 randomly selected pellets from each treatment on Days 3 and 11. On the first 2 days of the experiment sufficient numbers of eggs were found in all treatments to perform an egg hatching experiment. Eggs from each treatment and collection day were maintained separately in Nunclon 6-well multi-dishes containing filtered sea water. Hatched nauplii were counted after 3 and 5 d incubations.

Feeding experiment on *Calanus finmarchicus*. Specimens for the *Calanus finmarchicus* experiment were obtained on June 6 outside Syltefjordstauran (70° 35′ N, 30° 30′ E) in the southern Barents Sea. The samples were kept in 50 l polypropylene containers in darkness at 5°C and fed ad libitum on the diatom *Chaetoceros* sp. until the start of the experiment.

In the Barents Sea, *Calanus finmarchicus* have a 1-yr life cycle and thus only produce eggs in spring coupled with the spring bloom. As no egg production could be assessed due to the timing it was decided to run a different experiment on *C. finmarchicus* looking at the feeding ability after exposure to crude oil WSF for 11 and 18 d. A non-disturbing continuous flow-through system was constructed to expose *C. finmarchicus* stage CV continuously to crude oil WSF. Filtered sea water for the experiment was kept in a 300 l container in a climate room and cooled to 5.5 ± 1°C. On Days 5 and 9 the water container was re-filled resulting in a temperature increase of less than 1°C. Water was pumped through columns with oil covered beads which were prepared as previously described for the *C. glacialis* experiment, except that the glass beads were dried for only 24 h in room temperature in the *C. finmarchicus* experiment. Prior to the start of the experiment, the columns were flushed for 24 h to remove the most soluble crude oil components. The copepods were kept in 1 l blue cap bottles and the flow rate through the bottles was 1.7 ml min^{-1}. The flow rate through the columns containing oil-covered beads was 5.1 ml min^{-1} but this water was distributed to 3 sepa-

rate 1 liter blue cap bottles. (peristaltic pump; Watson Marlow 205U/CA).

Specimens were randomly added to 1 of the 3 treatments (CT, LT and HT) in blue cap bottles and exposed to WSF without food addition. After 11 and 18 d exposures, the ability of the copepods to feed was examined by ordinary algae clearance incubations. For each oil exposure treatment 6 copepods were transferred to individual 300 ml amber bottles prefilled with a suspension of 8800 (11 d exposure) and 14 400 (18 d exposure) cells ml^{-1} of *Chaetoceros* sp,. The algae were from a culture kept at 5°C in exponential growth phase on f/2 medium (Guillard & Ryther 1962) with silica addition. The bottles were kept in darkness for 24 and 72 h (11 and 18 d exposures, respectively) at 5°C and carefully turned several times during incubation to re-suspend the algae. Six replicates without copepods were also incubated to measure the growth of the algae during the incubation time. The experiment was terminated by taking 3 replicate samples of the algae contents for freezing and later quantified using a Fuchs-Rosenthal counting chamber (4 subsamples counted for each replicate algae sample). The contents of the bottles were then filtered through plankton netting (80 µm), the condition of the copepods was observed under a stereo microscope and fecal pellets were counted.

Composition and concentration of crude oil WSF. The chemical composition of crude oil differs from field to field and over time at individual fields. In addition, crude oil consists of thousands of different chemicals making it unfeasible to obtain full characterization of the chemical composition of a given crude oil. Instead the concentration of single compounds previously shown to cause detrimental effects on biota is used as an indicator. A commonly used indicator is the '16-EPA PAH' which consists of 16 PAHs chosen by the US Environmental Protection Agency (US EPA 1979). Water for PAH (16-EPA) analyses was collected on May 12, 18 and 22 for the *Calanus glacialis* experiment, and on June 27, and July 3 and 14 for the *C. finmarchicus* experiment. In both experiments samples were removed to 2 l amber bottles and immediately frozen at –20°C. These samples were later analyzed using the protocol EPA-8270-C to detect and quantify selected compounds using gas chromatography–mass spectrometry (GC–MS) (US EPA 1996). Analyses were performed by ALS Laboratory group.

Statistical analyses. Statistical analyses were run using Sigma Stat. Treatment differences in egg and fecal pellet production were analyzed by 1-way ANOVA. Egg hatching was evaluated using a 2-way ANOVA followed by a post-hoc Student-Newman-Keuls (SNK) test. In the *C. finmarchicus* experiment, treatment effects were evaluated by 1-way ANOVA followed by a post-hoc Tukey test.

RESULTS

Water soluble fraction of crude oil

In the *Calanus glacialis* experiment, treatment concentrations (16-EPA) on the first day were 10.4 µg l^{-1} (HT) and 3.6 µg l^{-1} (LT) (Fig. 1). Of the 16 PAHs screened, only 4 exceeded the detection limits of 0.01 to 0.1 µg l^{-1}; while the other 12 compounds may also have been present in WSF, they were not detected by GC–MS. The PAHs detected in both treatments were naphthalene, fluorene, phenanthrene and acenaphthylene. No oil components were detected in the control on any of the sampling days. Total concentrations of 16-PAH in WSF gradually decreased over time. This decrease was caused by a decrease in naphthalene, while the heavier PAH compounds were more stable. An important difference in PAH composition between HT and LT was that LT exhibited a much lower relative naphthalene concentration compared to HT (see Fig. 1).

In the *Calanus finmarchicus* experiment, the WSF (16-EPA) concentration gradually decreased from the initial concentrations of 7.0 (HT) and 3.4 µg l^{-1} (LT) (Fig. 2) with no oil components detected in the control. Again, a high proportion of naphthalene was found in both HT and LT groups but the amount of naphthalene decreased rapidly between the first 2 sampling dates. There was a modest increase in naphthalene and phenanthrene concentrations on Day 18.

Calanus glacialis

Egg and fecal pellet production

Egg production was monitored over 11 d and is given as average cumulative production per female (Fig. 3). In each treatment, 2 females did not produce eggs in this period. These were considered to be reproductively immature and were excluded from further analysis along with dead individuals (CT: 2 ind.; LT: 3 ind.; HT: 3 ind.). Specimens of females were thus reduced to 16, 15 and 15 in the CT, LT and HT, respectively. Daily egg production ranged between 0.1 to 11.8 eggs $female^{-1}$ d^{-1} with production rates decreasing during the experiment as reflected in the leveling-off of the slope of the cumulative egg production curve (Fig. 3). Females in the CT and LT groups produced more eggs than females in the HT group, but the difference among groups was not statistically significant (1-way ANOVA, $p > 0.05$).

Fecal pellet production is given as average cumulative number produced per female during the 11 d experiment (Fig. 4). There was no significant difference

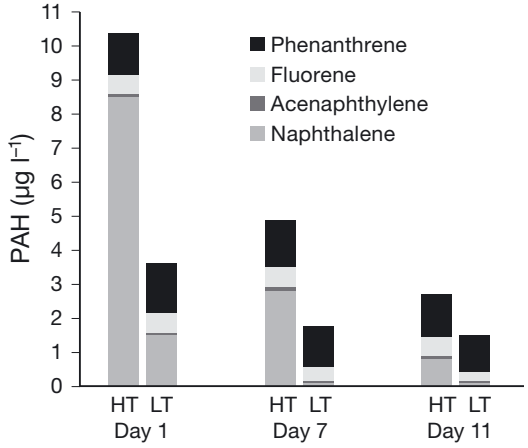

Fig. 1. Concentrations and composition of detectable crude oil polycyclic aromatic hydrocarbons (PAHs) in filtered seawater samples from *Calanus glacialis* feeding and reproduction experiment on 3 separate days. HT: high concentration treatment; LT: low concentration treatment

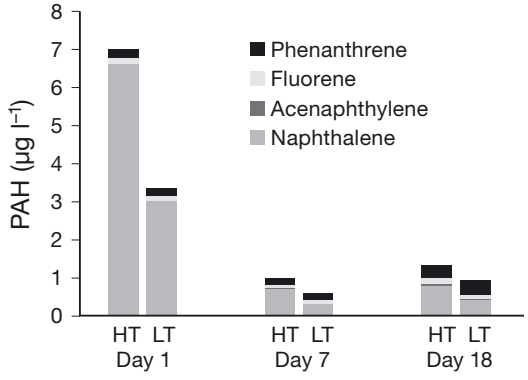

Fig. 2. Concentrations and composition of detectable crude oil polycyclic aromatic hydrocarbons (PAHs) in filtered seawater samples from *Calanus finmarchicus* feeding experiment on 3 separate days. HT: high concentration treatment; LT: low concentration treatment

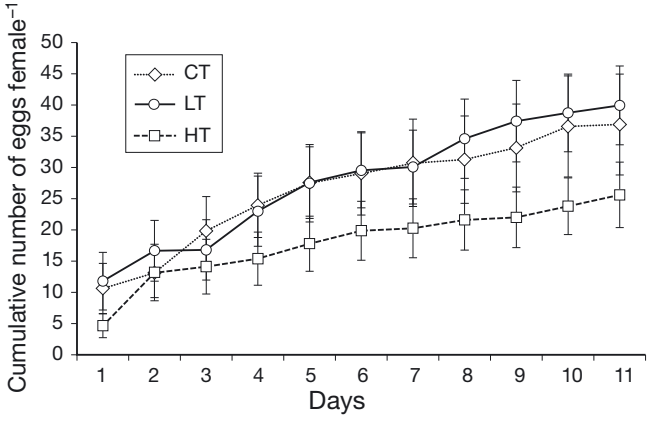

Fig. 3. *Calanus glacialis*. Mean (±SE) cumulative egg production per female during exposure to crude oil polycyclic aromatic hydrocarbons dissolved (PAHs) in filtered seawater. CT: control treatment (0 µg PAHs l^{-1}); LT: low concentration treatment (3.6 µg PAHs l^{-1}); HT: high concentration treatment (10.4 µg PAHs l^{-1})

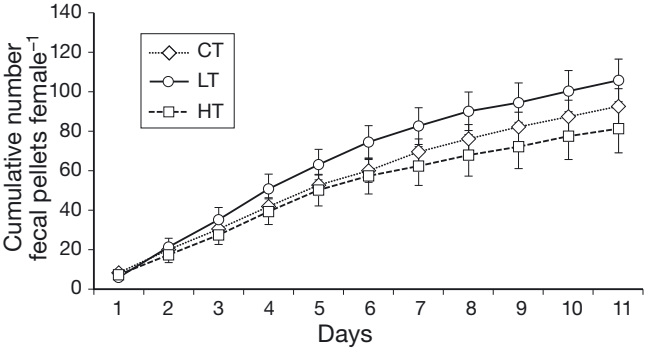

Fig. 4. *Calanus glacialis*. Mean (±SE) cumulative fecal pellet production during exposure to crude oil polycyclic aromatic hydrocarbons dissolved in filtered seawater. See Fig. 3 for treatment details

Table 1. *Calanus glacialis*. Reproductive success in terms of total number of eggs laid and hatched from females exposed to crude oil polycyclic aromatic hydrocarbons (PAHs) dissolved in filtered seawater on the first 2 days of the experiment. See Fig. 3 for treatment details

Treatment	Eggs laid	Eggs hatched	Replicates (n)
Day 1			
CT	170	115	7
LT	183	101	7
HT	94	11	8
Day 2			
CT	40	17	5
LT	75	44	5
HT	130	35	5

in fecal pellet production among treatments (1-way ANOVA, $p > 0.05$). Fecal pellet size also did not differ among treatments or treatment day (1-way ANOVA, $p > 0.05$).

Egg hatching

On the first 2 days of the experiment the number of egg-laying females as well as total number of eggs was sufficient and comparable to examine hatching success (Table 1). There was no significant difference in egg production between the 2 days (2-way ANOVA, $p > 0.05$) thus the combined hatching success is presented in Fig. 5. A statistically significant decrease in hatching success was determined for HT compared to CT (2-way ANOVA followed by post hoc SNK test, $p = 0.03$). There was no difference in hatching success between LT and CT (post hoc SNK test, $p > 0.05$).

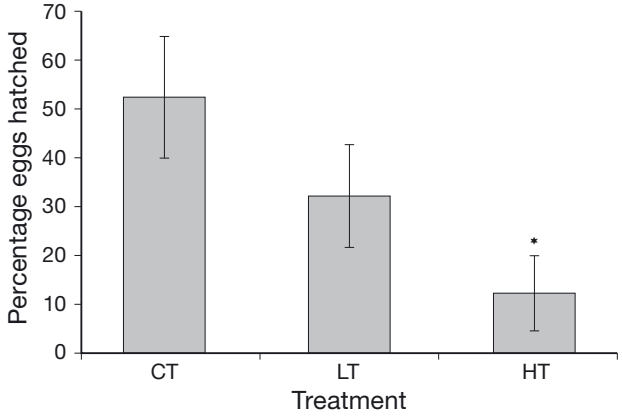

Fig. 5. *Calanus glacialis*. Mean (±SE) hatching success of eggs from females exposed to crude oil polycyclic aromatic hydrocarbons (PAHs) dissolved in filtered seawater. CT: control treatment (0 µg PAHs l^{-1}); LT: low concentration treatment (3.6 µg PAHs l^{-1}); HT: high concentration treatment (10.4 µg PAHs l^{-1}). *Significant difference from control ($p < 0.05$)

Calanus finmarchicus

The calculation of ingestion rate in algae clearance experiments relies on a decrease in algae concentration in bottles containing copepods compared to bottles without copepods (Frost 1972). However, in these experiments there was a higher increase in algae cell concentration in bottles containing copepods (Fig. 6), and hence meaningful (i.e. non-negative) ingestion rates could not be calculated. However, assuming that algae growth is affected similarly by oil-exposed and non-exposed copepods the algae concentrations in the bottles after 24 and 72 h indicate differences in feeding (Fig. 6). In the 11 d exposure experiment (24 h incubation) the number of algae cells found in HT was significantly higher than CT (1-way ANOVA followed by post hoc Tukey, $p = 0.02$) whereas there was no statistically significant difference between LT and CT (1-way ANOVA followed by post hoc Tukey, $p > 0.05$). In the 18 d exposure experiment (72 h incubation) algae cell number changes showed the same trends, but no differences between treatments were found (1-way ANOVA) (Fig. 6).

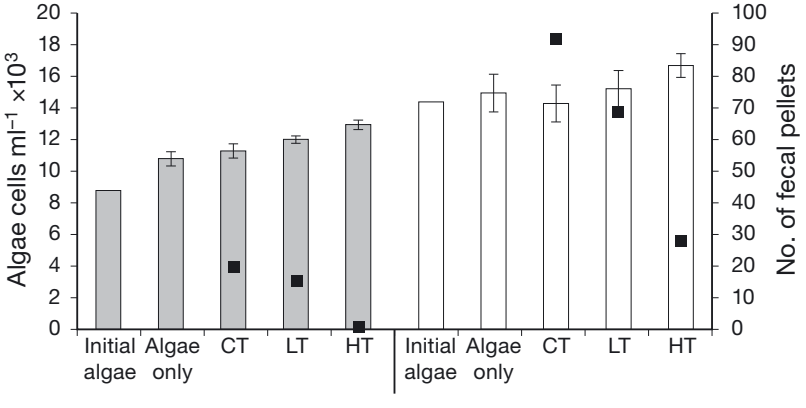

Fig. 6. Mean (±SE) initial concentrations of algae cells and concentrations found after 24 h (light grey bars) and 72 h (white bars) incubation with *Calanus finmarchicus* previously exposed to crude oil polycyclic aromatic hydrocarbons (PAHs) for 11 d (n = 6) and 18 d (n = 5), respectively. Black squares: mean number of fecal pellets produced in each treatment. CT: control treatment (0 µg PAHs l^{-1}); LT: low concentration treatment (3.4 µg PAHs l^{-1}); HT: high concentration treatment (7.0 µg PAHs l^{-1})

Fecal pellets production was highest in CT, lower in LT and lowest in the HT in both the 11 and 18 d feeding experiments, thus also suggesting a decreasing feeding rate with higher WSF concentration, but the differences were not significant (1-way ANOVA, $p > 0.05$).

DISCUSSION

Compositional changes in WSF during exposure of *Calanus*

In a natural oil spill the composition of oil will change over time as a result of evaporation, volatilization, emulsification, dissolution and oxidation, collectively known as 'weathering' (National Research Council 2003). Due to weathering, the lighter PAH compounds are removed faster from seawater compared to the heavier, and less soluble, compounds. As a result, the proportion of heavier compounds to the total PAH concentration will increase over time. In both experiments, the changes observed in the composition of WSF (Fig. 1) mimic nicely the expected changes in PAH composition associated with weathering processes after a natural oil spill. The reduction in total PAH was mainly associated with the elimination of naphthalene, while concentrations of the slightly heavier fluorene and phenanthrene remained constant throughout the experiments.

Both the concentration and composition of PAHs in WSF produced in a column system as employed here depends on several factors, including the amount of crude oil added to the beads, treatment of the beads after oil addition ('weathering process') and flow rate through the columns. Here the concentration of crude oil and the amount of beads in the columns were the same in the 2 experiments. However, it was decided to flush out some of the lighter compounds in the *Calanus finmarchi-*

cus exposure instead of the slower drying and evaporation employed in the *C. glacialis* experiment. In addition, the flow rate in the *C. finmarchicus* experiment was lower as this was a continuous flow through system where the flow rate had to be low enough to minimize the water use but high enough to maintain a healthy environment in the bottles. As a result, the final concentrations of PAHs in the 2 different experiments differed. In the *C. glacialis* experiment the copepods experienced a maximum of 10.4 µg PAHs l^{-1} while the highest concentration in the *C. finmarchicus* experiment was 7.0 µg PAHs l^{-1}. However the LT concentrations in the *C. glacialis* and *C. finmarchicus* experiments were comparable (3.6 and 3.4 µg l^{-1}, respectively). In the LT column for the *C. glacialis* experiment (most weathered), the naphthalene concentration was very low (42% of total PAH) while all other columns had 82 to 94% naphthalene (Day 1). Apparently naphthalene was rapidly removed from this column but remained in the similarly prepared HT column.

Data on concentrations of PAHs occurring in the water column following oil spills in cold waters are scare. Reddy & Quinn (1999) report a total water concentration of 5.6 µg l^{-1} for the same 4 PAHs registered in the present experiment, 7 d after the North Cape oil spill in 1996 (water temperature = 2°C). When compared with their results, the concentrations in the present experiment are not unrealistically high.

WSF influence on *Calanus glacialis* reproduction

We used egg production and hatching success as indicators of the consequences of exposure to WSF for *Calanus glacialis.* It should be noted that female specimens were not fed until the initiation of the experiment. *Calanus* species are clutch breeders and the interval between clutches varies with food availability and water temperature (Hirche et al. 1997). The number of clutches delivered by spawning females over the 11 d experiment varied from 1 to 5 (6 in one CT female). The average spawning interval (over 11 d) was 3.9 (CT), 4.8 (LT) and 5.1 (HT) d $female^{-1}$. These values are at the high end of the range previously reported for this species (1 to 6 d) (Hirche 1989). Food availability may also have resulted in decreased egg production during the experiment (0.1 to 11.8 eggs $female^{-1}$ d^{-1}), as fed females have been shown to have higher egg production rates (Hirche & Kattner 1993). Whether the overall egg production was limited due to the previous feeding history cannot be excluded, nor can the possibility that effects of the oil exposure may have been more pronounced with a different feeding history. However, *C. glacialis* is known to spawn when food is limiting, relying on lipid reserves for sustenance (Smith 1990) and all females were treated equally prior to the experiment, making the comparisons between treatments possible. We report no statistically significant difference in average cumulative egg production over the 11 d experiment among treatments (37 ± 8, 40 ± 6 and 26 ± 5 eggs $female^{-1}$ on Day 11 for CT, LT and HT, respectively). This indicates that egg production for *C. glacialis* is insensitive to exposure to WSF < 10.6 µg l^{-1}.

Egg hatching success was examined only for the first 2 experimental days due to lower egg production over time, as previously discussed. Hatching success was significantly lower in HT compared to CT, while there was no difference in hatching success in LT compared to CT.

The exposure of the eggs to PAHs may have been by direct exposure in the water just after being released or by vertical transfer from the female to the eggs. Eggs spawned by a female may have experienced direct exposure to WSF during the time period from the egg release from the female and egg retrieval from the different exposure beakers; a period of 0 to 24 h. However, Jensen et al. (2008) found a high hatching success in eggs exposed to 10 and 100 nM (2.02 µg l^{-1} and 20.23 µg l^{-1}) pyrene compared to the control, indicating no effect of direct exposure. This indicates that the membrane surrounding a copepod egg is protective against exposure to pyrene. We are not aware of other studies on copepod eggs showing that the membrane serves as a protective barrier against other PAHs. However, a number of studies conducted on fish eggs show that the membrane may serve as a protective barrier for some fish species, but not all, and that the protection varies with the PAH compound that eggs are exposed to (Stene & Lonning 1984, Carls & Rice 1988). Additional studies are needed to evaluate the vulnerability of copepod eggs in an oil spill scenario.

Exposure may also have occurred via vertical transfer from females to eggs resulting in a lower hatching success in HT eggs. During vitellogenesis 2, lipids are incorporated into eggs (Niehoff 2007) and PAHs bound to the lipids may be incorporated simultaneously. Vitellogenesis 2 overlaps in time with the final maturation of the eggs and the length of final maturation determines clutch interval. In this experiment, the clutch intervals were 3.9 to 5.1 d depending on treatment, and exposure time of the females producing eggs for the hatching experiment was 1 to 2 d. PAH accumulation occurs within a day in copepods (Duesterloh et al. 2002) so females did experience elevated levels of PAHs in the same time period as lipids are incorporated into the eggs and a vertical transfer of PAHs from female to eggs is possible.

WSF influence on *Calanus glacialis* feeding

Feeding of copepods may be assessed by fecal pellet production (Gaudy 1974), as clearance rate of prey items (Frost 1972), or by measuring gut fluorescence, digestive enzyme activity or grazing of labeled food items (Baars & Oosterhuis 1984). We used fecal pellet production as an indicator of the feeding response of *Calanus glacialis* to WSF.

The correct application of fecal pellet production as an indicator of feeding requires that copepods are exposed to similar food concentrations and produce fecal pellets of similar size (Jensen et al. 2006). To assess if the different exposure treatments resulted in differences in fecal pellet size, 20 pellets from each treatment were measured on 2 dates during the experiment. No differences in pellet size were found between treatments and time (1-way ANOVA, $p > 0.05$). Therefore fecal pellet production is considered a robust measure of feeding responses by exposed copepods.

There were no differences in the average cumulative number of fecal pellets produced per female in the different exposure groups. This indicates that exposure to PAHs does not affect feeding ability in *C. glacialis.* This contradicts studies performed on other *Calanus* species. Spooner & Corkett (1979) observed reduced feeding in *C. helgolandicus* exposed to WSF of Kuwait crude oil (2 µg l^{-1}) while Jensen et al. (2008) found increased feeding in exposed *C. glacialis* compared to controls after 9 d of exposure to 10 nM (2.02 µg l^{-1}) pyrene.

WSF influence on *Calanus finmarchicus* feeding

Stage CV of *Calanus finmarchicus* is the main overwintering stage found in the northern areas of distribution and CV dominates the population from early summer and onwards (Tande 1982). Stage CV copepods are smaller than the adult females but the lipid content is identical (Scott et al. 2000), i.e. they may be considered to be similarly vulnerable to contamination of lipophilic compounds such as PAHs.

The results from the current experiment show a clear indication of a potential response in feeding ability to longer term exposure to crude oil WSF in *Calanus finmarchicus.* In the 11 d experiment, the algae concentration found in the HT exposure was significantly higher than the concentration found in the CT (1-way ANOVA followed by post hoc Tukey, $p = 0.02$) while no difference was found between LT and CT (1-way ANOVA followed by post hoc Tukey, $p > 0.05$). The feeding experiment run on specimens exposed for 18 d showed the same trend but no difference between treatments (1-way ANOVA). The fecal pellet production verifies that feeding occurred, that it was higher in the CT compared to oil treatments and that HT specimens fed the least.

Addition of *Calanus finmarchicus* in the algae suspension gave a larger increase in algae numbers in CT, LT and HT treatment bottles compared to control bottles without copepods, therefore we were not able to quantify ingestion rates for this experiment. All incubation bottles were filled from a common mixture and copepods were randomly assigned to bottles to exclude the risk of imprecise algae addition. The increase in algae concentration was probably caused by additional nutrients provided by copepod excretion and leakage from algae cells due to sloppy feeding of the copepods (Moller et al. 2003, Zhang et al. 2006). It could further be argued that the copepods feeding the most, i.e. the control group, would supply more nutrients due to higher excretion and more broken cells and thus enhance algae growth further. Still, the number of cells found in the control treatment was significantly lower than in the high concentration treatment meaning that a higher growth of algae may have been compensated for by an even higher feeding rate.

In the present experiment *Calanus finmarchicus* was starved while exposed to WSF in filtered (5 µm) seawater, and then offered food in uncontaminated water. This means that the uptake of PAHs must have been by passive diffusion through the membranes. In contrast, Jensen et al. (2008) argue that pyrene uptake occurs primarily through ingestion of contaminated food as they found no effects on egg production for starved females but did find effects on fed females. Our results imply that there may even be a risk of contamination during the winter season when *Calanus* species in the high north are in diapause, i.e. in a non-feeding mode. Exposure of an over-wintering population to a blowout or other oil spill event may result in a reduction in feeding ability, hence negatively influencing the copepods' ability to acquire enough energy to go through the last molting to adults and egg production.

Comparison between species

The experiments on *Calanus glacialis* and *C. finmarchicus* were performed during different times of year and egg production was not assessed for *C. finmarchicus* because spawning only occurs during springtime in this species' northern range. In addition the feeding experiments for these 2 species were performed using 2 different approaches. This makes impossible a direct comparison of WSF exposure effects between the 2 species. However, the general finding that feeding behavior for the smaller and leaner species *C. finmarchicus* was negatively affected by PAH exposure while

C. glacialis was not affected agrees well with the results of Jensen et al. (2008). The exposure experiments conducted by Jensen et al. (2008) resulted in more severe effects of pyrene exposure on feeding and egg production for *C. finmarchicus* compared to *C. glacialis*. *C. finmarchicus* showed a decrease in average cumulative egg and fecal pellet production when exposed to 100 nM (20.23 µg l^{-1}) of pyrene compared to the control while *C. glacialis* exhibited an increase in average fecal pellet production on the last day of the experiment in the 10 nM (2.02 µg l^{-1}) treatment and no effects in the other concentrations or on egg production.

Differences in contaminant effects on individual animals may be related to several factors. The ability to metabolize PAHs varies highly among invertebrates (Rust et al. 2004) and even between sibling species (Bach et al. 2005). As no uptake, metabolism or depuration was measured in the current experiment it cannot be excluded that *C. finmarchicus* has a lower ability to metabolize PAHs than *C. glacialis.* Alternatively, the observed differences in responses may be related to larger lipid contents in *C. glacialis* (70% of dry weight [DW] in early autumn; Scott et al. 2000) compared to concentrations in *C. finmarchicus* (31% of DW in early autumn; Scott et al. 2000). Van Wezel & Opperhuizen (1995) hypothesized that lipophilic compounds bind to the hydrophobic part of cell membranes, creating a narcotic effect by disturbing normal cell functions. The higher amount of storage lipids in *C. glacialis* may bind lipophilic pollutants such as PAHs, leaving lower amounts of PAHs to interfere with cell membranes (Lassiter & Hallam 1990, Van Wezel & Opperhuizen 1995) and further lead to an increase in the time until observable effects and the concentration level for an observable effect.

CONCLUSION

These experiments show that we may detect sublethal life history effects on *Calanus* species at PAH concentrations relevant for an oil spill in the Arctic. A reduction in feeding as observed for *C. finmarchicus* and egg hatching success as observed in *C. glacialis* will have implications for copepod populations. When evaluating the impact of a contaminant it is thus appropriate to use changes in life history traits as these will appear prior to mortality and may have consequences for the ecosystem.

As seawater temperatures are predicted to increase in the Arctic (ACIA 2004), we also expect a change in the distribution and composition of the zooplankton community with a northward migration of populations (Beaugrand et al. 2002). In the Barents Sea this implies a shift from the more lipid-rich *Calanus glacialis* to the leaner *C. finmarchicus.* Based on the current knowledge a potential oil spill seems likely to affect *C. finmarchicus* populations more severely compared to *C. glacialis* populations, since exposure effects are detected in both earlier and later stages in the former species but only in younger stages in the latter species. The season of the year, i.e. during reproduction or not, as well as the residence time within a potential spill are important factors deciding the risk for populations. Oil-extraction activities are projected to increase in the Barents Sea, and taking into consideration the importance of these copepod species in the Arctic ecosystems (Loeng & Drinkwater 2007) more knowledge on the effects on both *Calanus* species, and why these effects may differ between species, is needed.

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LITERATURE CITED

ACIA (2004) Impacts of a warming Arctic: Arctic climate impact assessment. Cambridge University Press, Cambridge

Arnkvaern G, Daase M, Eiane K (2005) Dynamics of coexisting *Calanus finmarchicus*, *Calanus glacialis* and *Calanus hyperboreus* populations in a high-Arctic fjord. Polar Biol 28:528–538

Baars MA, Oosterhuis SS (1984) Diurnal feeding rhythms in North Sea copepods measured by gut fluoresence, digestive enzyme activity and grazing on labeled food. Neth J Sea Res 18:97–119

Bach L, Palmqvist A, Rasmussen LJ, Forbes VE (2005) Differences in PAH tolerance between *Capitella* species: underlying biochemical mechanisms. Aquat Toxicol 74: 307–319

Bambulyak A, Frantzen B (2009) Oil transport from the Russian part of the Barents Region. Status per January 2009. The Norwegian Barents Secretariat, Kirkenes, and Akvaplan-niva, Tromsø

Barata C, Calbet A, Saiz E, Ortiz L, Bayona JM (2005) Predicting single and mixture toxicity of petrogenic polycyclic aromatic hydrocarbons to the copepod *Oithona davisae*. Environ Toxicol Chem 24:2992–2999

Beaugrand G, Reid PC, Ibanez F, Lindley JA, Edwards M (2002) Reorganization of North Atlantic marine copepod biodiversity and climate. Science 296:1692–1694

Bellas J, Thor P (2007) Effects of selected PAHs on reproduction and survival of the calanoid copepod *Acartia tonsa.* Ecotoxicology 16:465–474

Camus L, Olsen GH (2008) Embryo aberrations in sea ice amphipod *Gammarus wilkitzkii* exposed to water soluble fraction of oil. Mar Environ Res 66:221–222

Carls MG, Rice SD (1988) Sensitivity differences between

eggs and larvae of walleye pollock (*Theragra chalcogramma*) to hydrocarbons. Mar Environ Res 26:285–297

Carls MG, Short JW, Payne J (2006) Accumulation of polycyclic aromatic hydrocarbons by *Neocalanus* copepods in Port Valdez, Alaska. Mar Pollut Bull 52:1480–1489

Chapman PM, Riddle MJ (2005) Polar marine toxicology—future research needs. Mar Pollut Bull 50:905–908

Cowles TJ, Remillard JF (1983) Effects of exposure to sublethal concentrations of crude oil on the copepod *centropages hamatus*. 1. Feeding and egg production. Mar Biol 78:45–51

Daase M, Vik JO, Bagoien E, Stenseth NC, Eiane K (2007) The influence of advection on *Calanus* near Svalbard: statistical relations between salinity, temperature and copepod abundance. J Plankton Res 29:903–911

de Maagd PGJ, van de Klundert ICM, van Wezel AP, Opperhuizen A, Sijm DTHM (1997) Lipid content and time-to-death-dependent lethal body burdens of naphthalene and 1,2,4-trichlorobenzene in fathead minnow (*Pimephales promelas*). Ecotoxicol Environ Saf 38:232–237

Duesterloh S, Short JW, Barron MG (2002) Photoenhanced toxicity of weathered Alaska north slope crude oil to the calanoid copepods *Calanus marshallae* and *Metridia okhotensis*. Environ Sci Technol 36:3953–3959

Falk-Petersen S, Hopkins C, Sargent J (1990) Trophic relationships in the pelagic, Arctic food web. In: Barnes M, Gibson RN (eds) 24th European Marine Biology Symposium, Oban, 4–10 Oct 1989. Aberdeen University Press, p 315–333

Fingas MF, Hollebone BP (2003) Review of behaviour of oil in freezing environments. Marr Pollut Bull 47:333–340

Frost BW (1972) Effects of size and concentration of food particles on feeding behavior of marine planktonic copepod *Calanus pacificus.* Limnol Oceanogr 17:805–815

Gaudy R (1974) Feeding of four species of pelagic copepods under experimental conditions. Mar Biol 25:125–141

Guillard RR, Ryther JH (1962) Studies of marine planktonic diatoms. 1. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. Can J Microbiol 8:229–239

Hirche HJ (1989) Egg production of the Arctic copepod *Calanus glacialis*: laboratory experiments. Mar Biol 103: 311–318

Hirche HJ (1997) Life cycle of the copepod *Calanus hyperboreus* in the Greenland sea. Mar Biol 128:607–618

Hirche HJ, Kattner G (1993) Egg production and lipid contents of *Calanus glacialis* in spring: indications of a food-dependent and food-independent reproductive mode. Mar Biol 117:615–622

Hirche HJ, Meyer U, Niehoff B (1997) Egg production of *Calanus finmarchicus*: effect of temperature, food and season. Mar Biol 127:609–620

Hjorth M, Dahllöf I (2008) A harpacticoid copepod *Microsetella* spp. from sub-Arctic coastal waters and its sensitivity towards the polyaromatic hydrocarbon pyrene. Polar Biol 31:1437–1443

Hylland K (2006) Polycyclic aromatic hydrocarbon (PAH) ecotoxicology in marine ecosystems. J Toxicol Environ Health 69:109–123

Jensen LK, Carroll J, Pedersen G, Hylland K, Dahle S, Bakke T (2006) A multi-generation *Calanus finmarchicus* culturing system for use in long-term oil exposure experiments. J Exp Mar Biol Ecol 333:71–78

Jensen MH, Nielsen TG, Dahllöf I (2008) Effects of pyrene on grazing and reproduction of *Calanus finmarchicus* and *Calanus glacialis* from Disko Bay, West Greenland. Aquat Toxicol 87:99–107

Koszteyn J, Timofeev S, Weslawski JM, Malinga B (1995) Size structure of *Themisto abyssorum* Boeck and *Themisto libellula* (Mandt) populations in european Arctic seas. Polar Biol 15:85–92

Lassiter RR, Hallam TG (1990) Survival of the fattest: implications for acute effects of lipophilic chemicals on aquatic populations. Environ Toxicol Chem 9:585–595

Lee RF (1974) Lipid composition of copepod *Calanus hyperboreas* from Arctic ocean: changes with depth and season. Mar Biol 26:313–318

Loeng H, Drinkwater K (2007) An overview of the ecosystems of the Barents and Norwegian Seas and their response to climate variability. Deep-Sea Res II 54:2478–2500

Maclean SF (1973) Life cycle and growth energetics of Arctic crane fly *Pedicia hannai antenatta.* Oikos 24:436–443

Melle W, Skjoldal HR (1998) Reproduction and development of *Calanus finmarchicus*, *C. glacialis and C. hyperboreus* in the Barents Sea. Mar Ecol Prog Ser 169:211–228

Møller EF, Thor P, Nielsen TG (2003) Production of DOC by *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus* through sloppy feeding and leakage from fecal pellets. Mar Ecol Prog Ser 262:185–191

National Research Council (2003) Oil in the sea III: inputs, fates, and effects. National Academy Press, Washington, DC

Niehoff B (2007) Life history strategies in zooplankton communities: The significance of female gonad morphology and maturation types for the reproductive biology of marine calanoid copepods. Prog Oceanogr 74:1–47

Olsen GH, Carroll ML, Renaud PE, Ambrose WG, Olsson R, Carroll J (2007a) Benthic community response to petroleum-associated components in Arctic versus temperate marine sediments. Mar Biol 151:2167–2176

Olsen GH, Sva E, Carroll J, Camus L and others (2007b) Alterations in the energy budget of Arctic benthic species exposed to oil-related compounds. Aquat Toxicol 83:85–92

Olsen GH, Carroll J, Sva E, Camus L (2008) Cellular energy allocation in the Arctic sea ice amphipod *Gammarus wilkitzkii* exposed to the water soluble fractions of oil. Mar Environ Res 66:213–214

Reddy CM, Quinn JG (1999) GC-MS analysis of total petroleum hydrocarbons and polycyclic aromatic hydrocarbons in seawater samples after the North Cape oil spill. Mar Pollut Bull 38:126–135

Rust AJ, Burgess RM, Brownawell BJ, McElroy AE (2004) Relationship between metabolism and bioaccumulation of benzo[a]pyrene in benthic invertebrates. Environ Toxicol Chem 23:2587–2593

Scott CL, Kwasniewski S, Falk-Petersen S, Sargent JR (2000) Lipids and life strategies of *Calanus finmarchicus*, *Calanus glacialis* and *Calanus hyperboreus* in late autumn, Kongsfjorden, Svalbard. Polar Biol 23:510–516

Scott CL, Kwasniewski S, Falk-Petersen S, Sargent JR (2002) Species differences, origins and functions of fatty alcohols and fatty acids in the wax esters and phospholipids of *Calanus hyperboreus*, *C. glacialis* and *C. finmarchicus* from Arctic waters. Mar Ecol Prog Ser 235:127–134

Smith SL (1990) Egg production and feeding by copepods prior to the spring bloom of phytoplankton in Fram Strait, Greenland Sea. Mar Biol 106:59–69

Spooner MF, Corkett CJ (1979) Effects of Kuwait oils on feeding rates of copepods. Mar Pollut Bull 10:197–202

Stene A, Lonning S (1984) Effects of 2-Methylnaphthalene on eggs and larvae of 6 marine fish species. Sarsia 69: 199–203

Tande KS (1982) Ecological investigations on the zooplankton community of Balsfjorden, Northern Norway: generation cycles, and variations in body weight and body content of

carbon and nitrogen related to overwintering and reproduction in the copepod *Calanus finmarchicus* (Gunnerus). J Exp Mar Biol Ecol 62:129–142

US EPA (1979) Water-related enviromental fate of 129 priority pollutants. Office of Water Planning and Standards, Office of Water and Waste Management, US Environmental Protection Agency, Washington, DC

US EPA (1996) Method 8270C, semivolatile organic compounds by gas chromatography/mass spectrometry, Rev. 3. Office of Solid Waste, US Environmental Protection Agency, Washington DC

Van Wezel AP, Opperhuizen A (1995) Narcosis due to environmental pollutants in aquatic organisms: residue-based toxicity, mechanisms, and membrane burdens. Crit Rev Toxicol 25:255–279

Varanasi U (1989) Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment. CRC Press, Boca Raton, FL

Zhang WC, Li HB, Xiao T, Zhang J, Li CL, Sun S (2006) Impact of microzooplankton and copepods on the growth of phytoplankton in the Yellow Sea and East China Sea. Hydrobiologia 553:357–366

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