



ORIGINAL RESEARCH ARTICLE

The short-term effects of crude oil on the survival of different size-classes of cladoceran *Daphnia magna* (Straus, 1820)[☆]

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Received 15 October 2014; accepted 16 October 2014

Available online 29 October 2014

KEYWORDS

Crude oil;
Pollution;
Daphnia magna;
Survival rate;
Size-class

Summary We studied the acute effects of crude oil on the size-class population structure of the cladoceran *Daphnia magna*. *D. magna* were tested in three size-classes: small (1.4 mm, SE = 0.013), medium (2.5 mm, SE = 0.026), and large (3.1 mm, SE = 0.022) with six concentrations of crude oil (10, 50, 100, 400, 600, and 1700 mg L⁻¹). The most important results of our experiment were as follows: (1) Crude oil had no significantly effect on *D. magna* below concentration 100 mg L⁻¹. (2) An increasing crude oil concentration above 100 mg L⁻¹ sharply decreased the survival of *D. magna*, (3) and survival varied among size classes. Being in contact with the concentration of 400 mg L⁻¹ and above, all cladoceran specimens died after 96 h.

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[☆] Funding for this research was provided by Institutional research funding IUT02-20 of the Estonian Research Council. The study has been also supported by the projects “The status of marine biodiversity and its potential futures in the Estonian coastal sea” No 3.2.0801.11-0029 of Environmental protection and technology program of European Regional Fund and “Applications of ecological knowledge in managing oil spill risk (OILRISK)” of Central Baltic INTERREG IVA.

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Peer review under the responsibility of Institute of Oceanology of the Polish Academy of Sciences.



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1. Introduction

The growing demand for oil products has increased the amount of crude oil entering to the aquatic environment caused by the accidents or regular commercial activities. Damaging effects of oil toxicity on various ecosystem elements have been increasingly reported since 1960s (Baker, 2001; McCauley, 1966; Peterson et al., 2003). The majority of studies have focused on the oil spill effects on large organisms such as macrophytes (Kotta et al., 2009; Leiger et al., 2012; Pezeshki et al., 2000), birds (Jenssen, 1994), fish (Carls et al., 1999) or marine mammals (Engelhardt, 1983). However, in order to better understand the effects of oil pollution to the whole

ecosystem, it is crucial to involve studies also on smaller taxa using a rigorous experimental frame.

To date, some studies have focused on the impacts of crude oil to plankton communities (e.g. Jung et al., 2012; Varela et al., 2006). Nevertheless, most of these studies have correlative nature and the reported oil spill effects are likely confounded by other environmental variables that are not covered by sampling design. As a consequence, the adverse effect of crude oil cannot often be distinguished (Batten et al., 1998; Hu et al., 2011). Moreover, most of the studies have not investigated the oil pollution induced responses of different life stages of planktonic organisms although the size of organisms is expected to modulate the responses to the intoxication of biota (Arzate-Cárdenas et al., 2011; Brooks et al., 2003; Kostial et al., 1978).

Cladocerans within the genus *Daphnia* are one of the key organisms in aquatic ecosystems being an essential link between primary production and many important fish species and at the same time exerting a strong control over phytoplankton abundance (Lampert, 1987). *Daphnia magna* is commonly found in brackish water (Arner and Koivisto, 1993) but also inhabits freshwater environments. Therefore, *D. magna* is acknowledged as an important test-organism in ecotoxicological studies both in fresh and brackish waters.

Our experiment focused on short-term effects of crude oil on the cladoceran *Daphnia magna* (Straus 1820) in order to assess the acute effects of crude oil on their survival rate. Furthermore, we explored a potential of different life stages of cladocerans to modulate the effect of intoxication. Previous studies quantified the crude oil effects mainly on the first developmental stages of *D. magna* (<24 h old in Martinez-Jeronimo et al. (2005); <48 h old in Ullrich and Millemann (1983); and <10 days in Ratushnyak et al. (2009)) and in one case also mature adults (Dowden, 1962).

The hypotheses of this study are: (1) As an opportunistic species *D. magna* is not influenced by very low concentrations of crude oil; (2) An increased crude oil concentration decreases the survival rate of *D. magna*; (3) Different developmental stages of *D. magna* have different sensitivity to crude oil, whereas the interactive effect of crude oil concentration and cladocerans' life stage may dominate over the separate effect of crude oil concentration.

2. Material and methods

2.1. Experimental organisms

D. magna specimens were obtained from continuous cultures maintained for several years at the Estonian Marine Institute of the University of Tartu. The experiments manipulating crude oil concentration and size-classes of the cladocerans were performed at the Estonian Marine Institute. The stock culture was maintained in 20 L aquarium and fed an ad libitum diet of *Scenedesmus obliquus*. The culture was kept in natural light conditions at room temperature ($20 \pm 2^\circ\text{C}$).

2.2. Experimental set-up

The cladocerans were separated into three size classes: small (1.4 mm, SE = 0.013; 3 days old), medium (2.5 mm, SE = 0.026;

6 days old), and large (3.1 mm, SE = 0.022; 9 days old). From each size-class 10 individuals were placed into 50 ml Erlenmeyer's flasks in four replicates at six concentrations of fresh ESPO blend crude oil (10, 50, 100, 400, 600, and 1700 mg L⁻¹). The concentrations were established as follows: (1) 1 g of crude oil was weighted using analytical balance with a precision of ± 0.001 g, (2) the crude oil was homogenized with water using Branson ultrasonic sonifier and (3) finally the required concentration was achieved by adding water. In order to minimize the stress to *D. magna*, we used the same water in the experiments where the culture was derived. Control flasks with no crude oil were also ran in four replicates.

When preparing the crude oil treatments in Erlenmeyer's flasks one half (25 ml) of the water was placed into flask with 10 specimens and another half (25 ml) was added a double concentration of the crude oil respective to the treatments. In addition, we measured experiment medium with Scasy Scårfe system particle counter to guarantee the sufficient food density for the cladocerans according to the literature (McMahon and Rigler, 1965; Schindler, 1968). We covered the test-flasks with aluminum foil to sterilize the test-medium and minimize the evaporation.

The prepared Erlenmeyer's flasks were placed on platform shaker Heidolph Unimax 2010 and run on the speed of 100 rpm. Although the oil emulsions were kept in suspension there was some accumulation in the surface layer. All the replicates were hold in test-conditions for 24 h at 20°C with a photoperiod of 16 h light and 8 h darkness.

2.3. Data analyses

After 24 h all incubated *D. magna* specimens were measured using binocular with ocular micrometer and their conditions were assessed. The cladocerans were counted as dead when they exhibited no movement after being touched with a needle. During measurements all individuals were treated gently to minimize the disturbance of incubated *D. magna* outside the experiment. After tallying the cladocerans, live specimens were placed back to the same conditions they were kept before the crude oil treatments. Every replicate sample was kept separately and measured after 48, 72, and 96 h from commencement of the tests.

The analysis of variance (ANOVA) was performed to separate the effects of size classes and crude oil concentration on the survival rate of *D. magna*. Bartlett's test was carried out prior to the analyses and the results confirmed the assumption of homoscedasticity. Post hoc Bonferroni tests were used to analyze which treatment levels were statistically different from each other (Sokal and Rohlf, 1981).

3. Results

All analyzed factors and interactions had a statistically significant effect on the survival of *D. magna* (Tables 1 and 2). Specifically, crude oil had no significantly effect on *D. magna* below 100 mg L⁻¹. Above this level, however, the increasing crude oil concentration almost linearly decreased the cladocerans' survival (Fig. 1). In addition, the experiment also demonstrated that the tolerance of *D. magna* to crude oil varied among cladocerans' size classes (Fig. 2). Although small- and large-sized cladocerans had relatively similar

Table 1 Two-way factorial ANOVA on the effect of crude oil concentration (1) and the size-class of *Daphnia magna* (2) on the survival rate of the cladoceran after 24 h.

Factor	Sum of squares	Degrees of freedom	Mean square	F	p-Level
Intercept	490,671.4	1	490,671.4	5195.345	0.000000
1	94,945.2	6	15,824.2	167.550	0.000000
2	2021.4	2	1010.7	10.702	0.000100
1 × 2	4211.9	12	351.0	3.716	0.000297
Error	5950.0	63	94.4		

responses (survival rates were 81%, and 79% respectively), medium-sized *D. magna* were significantly more vulnerable to the crude oil (survival rate 70%) (ANOVA post hoc Bonferroni $p_{\text{medium sized vs. other size groups}} < 0.05$). The median lethal concentrations (LC₅₀) at 24 h for small, medium and large size classes were 1025, 610 and 900 mg L⁻¹, respectively. At 96 h, however, the values were much lower at 210, 213 and 216 mg L⁻¹.

Furthermore, there was a significant interaction between cladoceran size and crude oil, i.e. different sized cladocerans responded differently on increasing crude oil concentration. The post hoc Bonferroni test indicated that most of the treatment levels above 100 mg L⁻¹ were statistically different after 24 h but not after 96 h (Figs. 3 and 4). Specifically, none of the cladocerans, being in contact with oil concentrations above 100 mg L⁻¹, showed recovering signs and died after 96 h even if placed back into their normal oil-free environment. In the control flasks all animals survived.

Above 100 mg L⁻¹ the survival rates of small- and large-sized *D. magna* decreased almost linearly with increasing oil concentrations: the large-sized specimens were more tolerant to the lowest dilution but their survival rate was decreasing more steeply with the raising oil concentration. However, medium size-class had lowest survival rates at all studied concentrations and declined nearly exponentially with increasing oil concentration.

4. Discussion

Our experiments supported the hypotheses that an increasing crude oil concentration decreases the survival of *D. magna* and the crude oil having different effect on each of the cladocerans' size-class was supported by current study. In contrast, the hypothesis that the interactive effect of crude oil concentration and the cladocerans' life stage may dominate over the

separate effect of crude oil concentration was not supported. We were also able to establish a threshold value of 100 mg L⁻¹ below which the effects of crude oil on the cladocerans was negligible. In our study the overall LC₅₀ values were considerably higher as compared to, e.g. Bobra et al. (1983). Such variation in LC₅₀ values may be attributed to differences in, e.g. test methodology, test duration and crude oil type.

The effects of oil pollution to plankton are complex involving many indirect and direct mechanisms. However, most effects are due to the increasing oil concentration. The indirect impact of oil pollution to plankton may result in the decrease of dissolved oxygen concentration and related degradation in water quality parameters (Harrel, 1985; Li and Boufadel, 2010; Neff and Stubblefield, 1995). Very high concentrations of crude oil may eliminate primary producers from the area, thus decreasing the food resource for heterotrophs (Chao et al., 2012; Karydis, 1982). On the other hand, species and populations respond differentially to oil pollution. Therefore, oil pollution may change the species composition by selectively eliminating the dominant grazers among plankton which may lead to the increasing abundance of primary producers (Miller et al., 1978) and intensified eutrophication process.

The current study focused mostly on direct damage and short-term effects of high (400–1700 mg L⁻¹) and low (10–100 mg L⁻¹) concentration crude oil on plankton survival. The used low concentrations are realistic of oil spill conditions (Bobra et al., 1989) whereas high concentrations are realistic in the case of emulsification process (Xie et al., 2007). The observed effects of high concentrations are plausibly due to the direct impact of oil on zooplankton, e.g. through inhibiting effect on glutamic oxalacetic transaminase activity (Biesinger and Christensen, 1972), gas-exchange inhibition (Pezeshki et al., 2000), and also direct feeding and absorption of oil and its residues by the organism (Duesterloeh et al., 2002). Besides, the chemoreception used by zooplankton during

Table 2 Two-way factorial ANOVA on the effect of crude oil concentration (1) and the size-class of *Daphnia magna* (2) on the survival rate of the cladoceran after 96 h.

Factor	Sum of squares	Degrees of freedom	Mean square	F	p-Level
Intercept	254,100.0	1	254,100.0	11,040.21	0.000000
1	190,850.0	6	31,808.3	1382.02	0.000000
2	50.0	2	25.0	1.09	0.343730
1 × 2	350.0	12	29.2	1.27	0.260263
Error	1450.0	63	23.0		

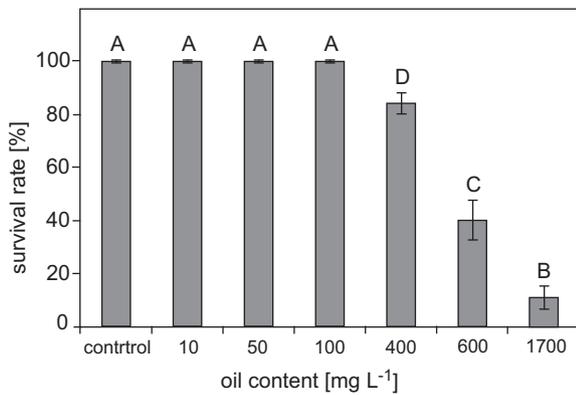


Figure 1 The effect of crude oil on the survival rate of *Daphnia magna*. Bars represent the mean \pm SE from all size-classes. The letters above the bars indicate significantly different groups (post hoc Bonferroni test).

foraging and mating may be also misled by crude oil soluble fraction (Herbert and Poulet, 1980). More importantly, crude oil has been also proven to have influence on live tissues, cells, and genetic material (Bhattacharjee and Fernando, 2008; Carls et al., 1999; Parab et al., 2008) which may interrupt the operation of physiological and biochemical system (Wezel and Opperhuizen, 1995) to the level that the photo-oxidation process can even take place at low concentrations (Karydis, 1982).

In our study we observed that crude oil destructively influenced the somatic structure of cladocerans, sometimes removing the whole carapace of the animal. This is likely due to the damaging effect on the parts standing for connecting the carapace to residual body. The survival rate was also influenced by the insoluble surface layer of crude oil which immobilized some of the specimens that had moved up to surface, unable to move their appendages (Fig. 5). To date, most of the laboratory studies have focused on the water soluble components of crude oil (Bhattacharjee and Fernando, 2008; Duesterloh et al., 2002; Martinez-Jeronimo et al., 2005). Focused research on the insoluble layer of

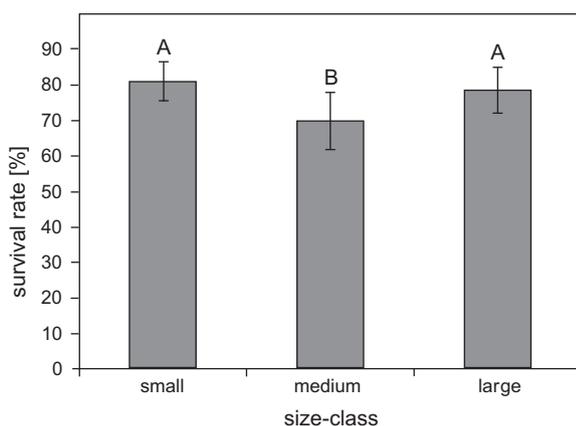


Figure 2 The effect of cladoceran size-class on the survival rate of *Daphnia magna*. Bars represent the mean \pm SE from all crude oil concentrations. Letters indicating the results of post hoc Bonferroni test. Different letters show different statistical groups.

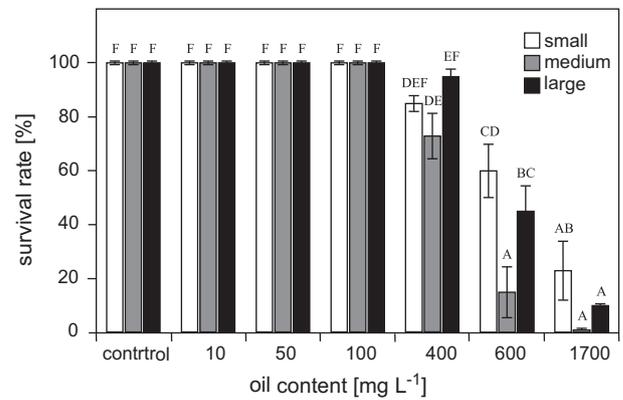


Figure 3 The interactive effect of cladoceran size-class and crude oil concentration to the survival rate of *Daphnia magna* after 24 h. Bars represent the mean \pm SE. Letters indicating the results of post hoc Bonferroni test. Different letters show different statistical groups.

crude oil would allow more thorough and generic conclusions about the oil pollution effects. Nevertheless, we believe that the water-soluble components may still be the key-factors to the cladocerans' survival. Likewise in our experiment the impact primarily increased with raising oil concentration regardless of the insoluble layer of oil present at every concentration tested.

Our experiment also demonstrated that all *D. magna*, which came into a contact with crude oil at concentrations below 100 mg L⁻¹ had a promising recovery. However, above this threshold value, all cladocerans died a few days after transferring to clean water. Thus, it is also possible in natural conditions that populations of cladocerans may not recover after coming into a short term contact with highly concentrated spills. Moreover, the life span of cladocerans is relatively brief (ca 25–100 days in MacArthur and Baillie, 1929) which leaves the impacted individuals with sufficiently short recovering time. It has been shown that cladocerans may not recover after severe disturbance of the environment

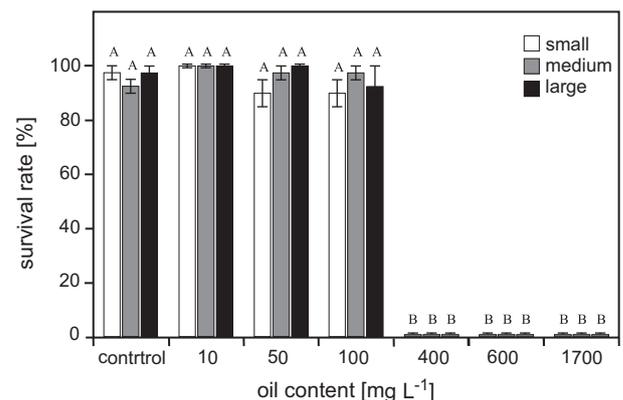


Figure 4 The interactive effect of cladoceran size-class and crude oil concentration to the survival rate of *Daphnia magna* after 96 h. Bars represent the mean \pm SE. Letters indicating the results of post hoc Bonferroni test. Different letters show different statistical groups.

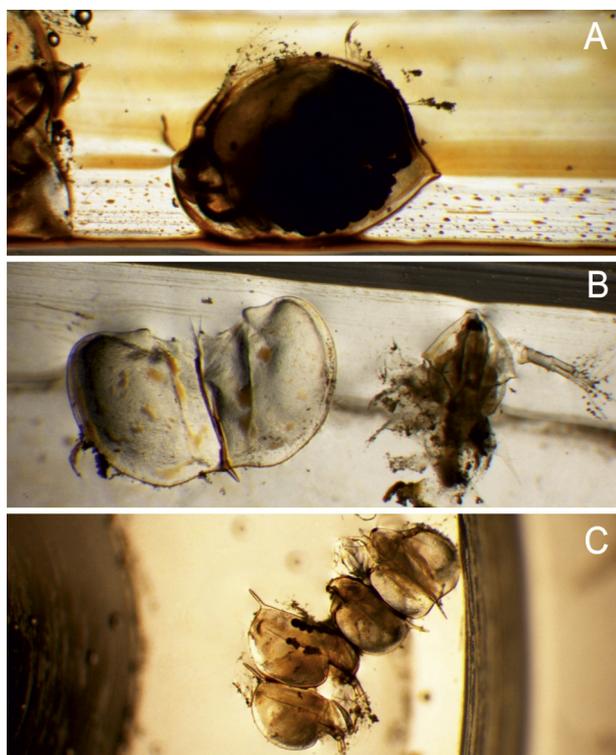


Figure 5 The appearance of *Daphnia magna* after oil pollution. (A) A specimen immobilized by the insoluble surface layer of crude oil. (B) Whole carapace of an animal removed as a result of oil pollution. (C) Cladocerans stick together due to crude oil.

(Yan et al., 2004); however, they have a potential to hatch from diapausing eggs which can survive more than 125 years and may be found up to 100 eggs per square meter of sediment (Cáceres, 1998). Nevertheless, the recovery process may be slow and challenging since the cladocerans are more vulnerable than other dominating zooplankton, e.g. copepods. Further, their attempt to recolonize may be counteracted by fish feeding (Yan et al., 2004). Thus, beside the direct chronic effects, the oil pollution may actually postpone the pelagic succession of the ecosystem.

The impacts varied among the cladoceran size classes. This suggests that, besides causing the increased mortality, oil spills may also modify population at size-class structure level. Despite that large-sized specimens tolerated low-concentration spills better than other size-classes the small-sized *D. magna* had the highest overall survival. Contrary, the medium-sized cladocerans were most vulnerable being almost died out at the greatest studied concentration. Although data on the effects of toxins on different size classes of cladoceran is limited, some authors (Hoang and Klaine, 2007; Muyssen and Janssen, 2007) report younger cladocerans to be more sensitive to toxins than older. Such elevated sensitivity of juveniles is likely due to age specific antioxidant activity in *D. magna* (Arzate-Cárdenas et al., 2011).

In our experiment we observed that among the studied size groups the medium-sized cladocerans were the most sensitive to the crude oil pollution. It is possible that *D. magna* is most active at the adolescent stage presented

by medium-size group and uses more energy speeding up the metabolic activities. Although it has been claimed that the metabolic rate decreases with age (Conceição et al., 1998; Fidhiany and Winckler, 1998), alternative evidence is likely to be available (Pérez-Camacho et al., 2000; Sukhotin et al., 2002). Thus, is possible that an elevated sensitivity of medium-sized cladocerans is due to increasing toxicity gained by an increasing metabolic rate at that life stage (Barry et al., 1995).

Such size-specific response of cladocerans to oil pollution needs to be considered when, e.g. modeling (Gin et al., 2001) or assessing the environmental impacts of oil spills in marine ecosystems. In nature, oil forms a thick surface layer which starts dispersing to deeper layers of water due to hydrodynamic forces (Chapman et al., 2007). The diffusion process is amplified in many cases by dispersants used in oil pollution which gains the toxicological effect to plankton and other marine life (Fisher and Foss, 1993; Singer et al., 1993). Different circumstances of oil pollution have varying effects either at size-class or the whole population levels, e.g. lower concentrations influence more phyto- and microzooplankton whereas higher concentrations have greater effects on mesozooplankton (Davenport et al., 1982) with medium size classes being mostly impacted (our experiment). Such size-class specific peculiarity has to be taken into account if making prevention or recovering proceedings, thus the reconsideration of oil pollution arrangements and standards is needed.

Acknowledgements

We thank Kalle Olli who kindly permitted to use his laboratory at the University of Tartu.

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