

Toxicity of Prudhoe Bay Crude Oil to Alaskan Arctic Zooplankton

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ABSTRACT. Bioassay experiments were conducted to determine the relative susceptibilities of three arctic zooplankton species to oil pollution, and the results were compared with the effects of an actual oil spill on a pond near Barrow. In both the bioassays and the pond, the addition of Prudhoe Bay crude oil was toxic to fairy shrimp (*Branchionecta paladosa* O. F. Müller), which seemed most sensitive, *Daphnia middendorffiana* Fischer, which was next most susceptible and *Heterocope septentrionalis* Juday and Muttkowski, which appeared somewhat resistant to the effects of oil. Cyclopoid copepods were the only common zooplankters able to survive the pond oil spill, and these were still present two and one half weeks after the spill. The rapid deaths of the other species, especially the branchiopods, suggest that zooplankton may be the most susceptible of all arctic freshwater organisms to oil pollution.

RÉSUMÉ. On a réalisé des expériences analytiques biologiques pour déterminer les susceptibilités relatives de trois espèces de zooplancton arctique, à la pollution pétrolière et on a comparé les résultats avec les effets d'un écoulement actuel de pétrole sur un étang près de Barrow. Dans les deux cas, l'addition du brut de Prudhoe Bay était toxique à la petite crevette (*Branchionecta paladosa*), qui semblait la plus sensible devant *Daphnia Middendorffiana* et *Heterocope septentrionalis* qui apparaissaient quelque peu résistantes aux effets du pétrole. Seuls, les copepodes cyclopoïdes, dans le zooplancton ordinaire, survivaient à l'écoulement de pétrole dans l'étang, deux semaines et demi après l'écoulement. La mort rapide des autres espèces, en particulier les branchiopodes, suggère que le zooplancton peut être le plus susceptible de tous les organismes arctiques d'eau douce, à la pollution du pétrole.

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INTRODUCTION

Due to the dramatic consequences of the rupture of oil tankers and the resulting massive environmental impact, much work has been done on the effects of oil pollution on marine ecosystems (Goldacre, 1968; Burns and Teal, 1971; Straughan, 1971; Chan, 1972). Similarly, the discovery and exploitation of arctic oil deposits in Alaska and Canada have led to considerable work on the potential effects of oil spills on arctic freshwater ecosystems (Barsdate *et al.*, 1973; Hutchinson and Hellebust, 1974; Rosenberg and Snow, 1975; Shindler *et al.*, 1975). However, because much of this work has focused on effects on phytoplankton (Kauss and Hutchinson, 1975; Soto *et al.*, 1975; Miller *et al.*, 1977; Federle *et al.*, in press), almost all research on the effects of oil on aquatic invertebrates comes from marine studies.

Moore and Dwyer (1974) list five responses of individual organisms to oil pollution: 1) direct lethal toxicity; 2) sub-lethal disruption of physiological activities; 3) effects of direct coating; 4) incorporation of hydrocarbons in food chains; and 5) changes in biological habitats. From extensive research with marine invertebrates, direct toxicity has been consistently identified as very important (Kontogiannis and Barnett, 1973; Barnett and Kontogiannis 1975;

Linden, 1976), although some work shows the importance of sub-lethal effects (Percy and Mullin, 1977) and the direct incorporation into marine food chains (Conover, 1971).

All research in the Alaskan arctic has shown freshwater zooplankton to be extremely sensitive to exposure to Prudhoe Bay crude oil. In an early experimental oil spill on a pond at the NARL laboratory in Point Barrow, 10 l of oil per metre square completely eliminated most, if not all, zooplankton from that pond (Federle *et al.*, in press). In this paper, results from aquaria bioassays and a pond spill are reported to demonstrate that spill levels more than an order of magnitude less than that of 1970 have the same impact on arctic zooplankton. Thus it seems freshwater arctic zooplankton may be more sensitive to oil pollution than any other arctic freshwater organisms.

METHODS

In the summer of 1975 an experimental oil spill was planned in pond omega (Miller *et al.*, 1977) at Barrow in arctic Alaska, with another pond (pond C; Federle *et al.*, in press) serving as a control. We performed a series of bioassay experiments to determine the relative susceptibilities of three common arctic freshwater zooplankton species to oil pollution, and the results were compared with the effects of the actual spill on these same species in pond omega.

The experimental bioassays to determine the degree of toxicity of Prudhoe Bay crude oil to *Daphnia middendorffiana*, *Branchionecta paladosa*, and *Heterocope septentrionalis* were all run in a similar fashion. Ten animals were isolated from pond C and placed in 250-ml Plexiglas cylinders to which no. 20 plankton netting had been affixed to both ends, with one end arranged so it could easily be removed to add animals and microscopically examine them. When filled with animals, the cylinders were introduced into the experimental aquaria. To overcome the problem of air being trapped by the netting while we were attempting to submerge the chambers in the test aquaria, the air was sucked through the netting with a large basting syringe. Each test aquarium received 2 chambers of 10 animals each. To each 20-l aquarium was affixed a glass plate about 1/3 way along the length of the aquarium and extending from the top to about half way to the bottom. With this arrangement, oil could be introduced to the larger area (538 cm²) and the chambers could be removed for examination through the smaller area without physical contact with the surface oil slick.

The aquaria were arranged on a table outdoors just to the east of the NARL laboratory at Point Barrow. Each aquarium was very gently aerated and a small amount of dense phytoplankton mixture from a fish aquarium was added to each aquarium every 2 days. Prudhoe Bay crude oil was added to each aquarium at the beginning of an experiment. The basic dose (120 ml/m²) was calculated to be one-half the experimental pond spill (240 ml/m²), and concentrations five times greater and five times less than this amount were

also used. Thus the one times dose amounted to 6.6 ml/aquarium (120 ml/m² or 0.33 ml/l), the five times dose was 33 ml/aquarium (600 ml/m² or 1.65 ml/l) and the 1/5x dose was 1.31 ml/aquarium (24 ml/m² or 0.06 ml/l). Federle *et al.* (in press) estimate from 14 to 21% of Prudhoe Bay crude oil of 0.877 specific gravity is potentially water soluble under the conditions likely in the test aquaria. If this estimate is accurate then the dose levels used would have resulted in 200-300 mg/l in the water-soluble fraction (WSF) at the five times dose, 40-60 mg/l in the WSF in the one times dose, and 7.4-11 mg/l in the WSF in the 1/5x dose. Since the floating slicks in the aquaria did not appear to diminish greatly, assuming much greater percentages would seem unwarranted. Unfortunately no measurements were possible at the time of the experiments. Each experiment had a control aquarium to which no oil was added. The same aquarium was used as a control throughout the experiments to assure that it was not contaminated; after use in one experiment the other aquaria were washed with detergent, rinsed with benzene, rinsed with acetone, washed again with detergent, carefully rinsed and dried and then re-used for further bioassays. The dose they received was independent of past use.

Throughout most of the experiments mortality in the chambers was examined daily. There was generally no doubt about an animal's condition because they all swam actively until succumbing and then generally lay completely quiet on the chamber bottom. Occasionally motionless *D. middendorffiana* could still be seen to have beating hearts; these were counted as alive until the heartbeat ceased. Individuals of the other two species were considered dead if motionless and unresponsive to prodding (Berdugo *et al.*, 1977).

Using a 2.35 l clear plastic Van Dorn water bottle suspended from a 2.5 m long pole, multiple zooplankton samples were taken from pond omega and pond C throughout June and July of 1975. Each sample was poured through a no. 25 plankton net suspended from a stand near the edge of the pond. Samples were taken from various areas and depths of the pond, and from 3 to 7 samples were combined and the animals preserved by mixing 95% alcohol with 8% formalin added into the sample on a one-to-one basis. After the experimental oil spill in which was spilled 240 ml/m² of Prudhoe Bay crude oil on pond omega on July 9, 1975, samples were taken in a similar manner but care was taken not to dip the sampler directly into the oil slick. Throughout this time samples were also taken from pond C to serve as a control.

RESULTS

The results of the aquarium bioassays of oil toxicity were quite straight-forward. The bioassay with *D. middendorffiana*, begun on 7 July 1975, showed that all concentrations of oil tested killed this animal within 120 hours (Fig. 1). Survivorship in the controls was excellent, with only 2 animals lost on the last day of the experiment. All dose levels appeared equally toxic, for the animals died at about the same rate each time. On 27 July 1975 a similar bioassay was performed using dose levels of 1, 1/5 and 1/25; in this

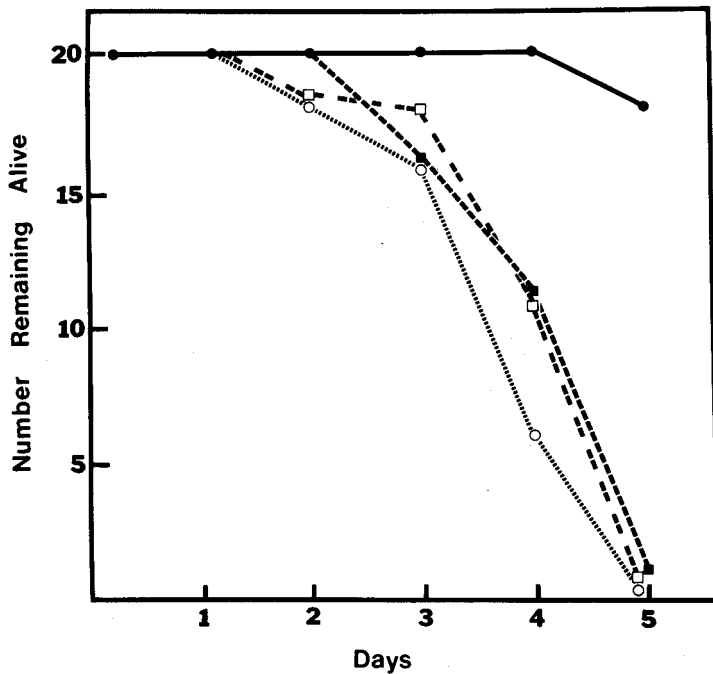


FIG. 1. Oil toxicity bioassay using *Daphnia middendorffiana* at 3 dose levels of Prudhoe Bay crude oil. The solid line connecting solid circles represents survivorship in a control aquarium to which no oil was added. The dashed line connecting solid squares represents survivorship of the one-fifth dose (1.3 ml/aquarium). The dotted line connecting open circles represents survivorship of the one times dose (6.6 ml/aquarium). The dashed line connecting open squares represents survivorship of the five times dose (33 ml/aquarium).

TABLE 1. Results of an oil bioassay with *Daphnia middendorffiana* at low dose levels of Prudhoe crude oil. The control received no oil, with 1x dose receiving 6.6 ml, 1/5x received 1.2 ml, and 1/25x received 0.24 ml of oil

Days	Aquarium D (Control)	Aquarium C (1/25)	Aquarium A (1/5)	Aquarium B (1)
0	20	20	20	20
1	20	20	20	20
2	20	20	20	20
3	20	20	20	20
4	20	20	20	19
5	—	—	—	—
6	—	—	—	—
7	20	20	15	15

experiment the 1/25 dose was not at all toxic after 168 hr (Table 1) and the 1 and 1/5 dose levels were somewhat less toxic than previously.

The fairy shrimp, *Branchionecta paladosa*, proved even more sensitive to exposure to WSF of oil. On 14 July 1975 an experiment identical to the *D. middendorffiana* bioassays was begun. Those animals exposed to the five times dose rapidly succumbed with all 20 dead within 48 hours (Fig. 2). At the one times dose level all died by 120 hr, and at the one-fifth dose level all died by 168 hrs. Once again the control survivorship was good, with 14 animals out of 20 surviving the full 192 hours of the experiment (Fig. 2).

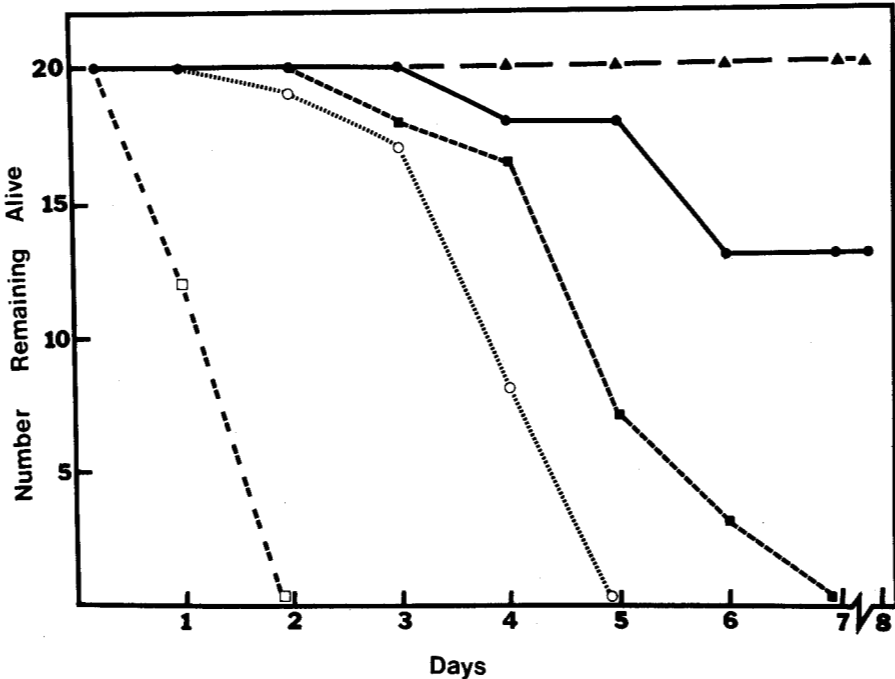


FIG. 2. Oil toxicity bioassay using fairy shrimp, *Branchionecta paladosa*, at three dose levels of Prudhoe Bay crude oil. The solid line connecting solid circles represents survivorship in a control aquarium to which no oil was added. The dashed line connecting solid squares represents survivorship of the one-fifth dose (1.3 ml/aquarium). The dashed line connecting open squares represents survivorship of the five times dose (33 ml/aquarium). The large dashed line connecting solid triangles represents survivorship of *Heterocope septentrionalis* placed in the chambers from the five times dose at day three.

At the 72 hour mark of this same experiment the now empty five times dose chambers were filled with 8 *Heterocope* and the chambers returned to the five times dose level. All of these animals lived 120 hr. On 22 July 1975 *Heterocope septentrionalis* was used for a complete bioassay at all three dose levels; however, interpretation became difficult due to fairly high mortality after just 2 days even in the controls, likely due to cannibalistic predation. Clearly, a voracious predator such as this copepod must be handled differently to determine its susceptibility to oil pollution.

The results of the bioassays suggest that three of the four groups of zooplankton dominant in the Barrow pond ecosystems and important throughout arctic Alaska (fairy shrimp *Branchionecta paladosa*, *D. middendorffiana*, and *Heterocope septentrionalis*) have different degrees of sensitivity to exposure to crude oil spills in lakes and ponds. The rapid mortality of fairy shrimp at the five times dose level suggests they are likely the most sensitive. *Daphnia middendorffiana* is next most sensitive with all individuals dead at all three dose levels within 120 hrs. As *Heterocope* were able to survive the five times dose level in the modification of the fairy shrimp bioassay, they may be least sensitive of the three zooplankton groups tested. Due to constraints of time cyclopoid copepods were not tested in the bioassay experiments.

The differential sensitivity of various species found in the Barrow oil bioassays can be compared to the sequence in which these species died and disappeared from pond omega in the Barrow oil spill experiment. As shown in Table 2, fairy shrimp disappeared very soon after the oil spill was introduced at 14:00 on 9 July 1975. Most of the fairy shrimp were dead 24 hr later and by the following day all were dead. *Daphnia middendorffiana* fared somewhat better, with some individuals living 3 days (Table 2). Some individuals of *Heterocope* lasted 6 days, but none could be found after 16 July (7 days). The cyclopoid copepods were by far the most resistant to oil toxicity with some animals captured alive two and one-half weeks after the experimental oil spill. Thus the differential sensitivities of the three species used in bioassay experiments corresponded to the sequence of disappearance from pond omega after the spill. Samples taken from pond C throughout July showed that all four groups remained in this control pond; fairy shrimp densities did decline after 20 July, whereas densities of *D. middendorffiana*, *Heterocope septentrionalis*, and *Cyclops* spp. all remained similar to those found in pond omega prior to the experimental oil spill throughout July.

DISCUSSION

Both the experimental pond spill and bioassay experiments indicate a consistent sequence of sensitivity to oil toxicity among common arctic freshwater zooplankton. Fairy shrimp are most sensitive followed in turn by *D. middendorffiana*, *Heterocope*, and finally *Cyclops* spp. which appear to be very resistant to exposure to oil. Others observed this same sequence. Barsdate *et al.* (1973) noted a decrease in zooplankton density, particularly of

WSF of Prudhoe crude oil toxic to arctic marine amphipods with the paraffinics somewhat more toxic. Until the toxic component(s) of the WSF are determined, precise definition of what constitutes a toxic dose is impossible.

However, there is good agreement with other reported work on freshwater invertebrates that the ranges used in the present study are toxic. Federle *et al.* (in press) found Prudhoe Bay crude toxic to arctic fairy shrimp and *D. middendorffiana* at 0.6 ml/l, which is twice the one times dose of the present study. Atlas and Busdosh (1976) found these same two groups of animals to succumb rapidly to exposure to the WSF of Prudhoe Bay crude at concentrations about five times greater than that used by Federle *et al.* (in press).

Concentrations of the WSF found to be toxic for marine invertebrates have varied widely with the type of oil used and the species of animal studied. Barnett and Kontogiannis (1975) found total mortality of a harpacticoid copepod within three days at a dose level of 1 ml diesel oil per liter, which is slightly less than the five times dose level used in the present study. They state that the critical level below which virtually no mortality occurred is 0.1 ml diesel oil per liter. Other work, generally expressed as mg/l of the WSF, has shown much lower levels of toxicity. Berdugo *et al.* (1977) observed considerable zooplankton mortality in three days with a WSF concentration of less than 1 mg/l. This is within a range at which Lee *et al.* (1977) found southern Louisiana crude oil was toxic to two species of amphipods. These low concentrations were only tested in the present study with the 1/25 dose which was not toxic to *D. middendorffiana* over seven days (Table 1).

We performed a similar series of bioassays at Toolik Lake (Miller and Hobbie, 1976) in July 1976 using *Daphnia middendorffiana*, *Holopedium gibberum*, *Zaddach* and *Heterocope septentrionalis* with Prudhoe Bay crude oil at doses similar to those reported here and found either no or very low toxicity. Initially we thought that use of a different batch of oil was responsible for these unexpected results. However, this explanation was discounted when we repeated the experiments at the dose levels used in 1975 with a sample of oil used by Federle *et al.* (in press) and again found no or very low toxicity, whereas this same oil had proved quite toxic to *D. middendorffiana* and fairy shrimp in experiments run just prior to this time in Barrow (Federle *et al.*, in press). They found no living zooplankton in 18-liter subponds after 3 days exposure to a dose level of 240 ml/m², whereas we could show no toxic effects with *D. middendorffiana* in aquaria at similar and five times greater dose rate.

The only major alteration in the design of the 1976 bioassay experiments was aeration. The aquaria were very vigorously aerated in the 1976 experiments at Toolik Lake whereas aeration was very much less in the bioassays in 1975 at Point Barrow. Anderson *et al.* (1974) has found that aeration of oil-in-water dispersions resulted in a loss of 80-90% of the aqueous hydrocarbons in 24 hr and that alkanes disappeared from the dispersions more rapidly than aromatics. Interestingly, although aromatic WSF are thought to

be the most toxic component, Busdosh and Atlas (1977) found the alkane fraction of Prudhoe Bay crude oil to be the most toxic to a marine amphipod. Rossi *et al.* (1976) found that 4 hr of aeration greatly reduced toxicity of WSF of four test oils to marine polychaetes. Although further research is needed, these results at least point to the possibility of aeration as an important mechanism for reducing toxicity of crude oil to aquatic invertebrates.

There seems little doubt that freshwater zooplankton are very sensitive to exposure to oil and in fact may be one of the most sensitive components of arctic aquatic ecosystems to oil pollution. Such sensitivity of zooplankton to oil pollution is very important from two points of view. First, in a lake the zooplankton make up an important food group for most arctic fish species (O'Brien, *et al.*, in press); therefore, fish which survive an oil spill on a lake may starve because of the elimination of their zooplankton food sources. As if to compound this problem, most arctic zooplankton have univoltine life cycles (Edmondson, 1955; Stross, 1969). Federle *et al.* (in press) and others have shown that while phytoplankton may be quite sensitive to exposure to oil, some species soon recover. With zooplankton, however, recovery may be very slow; if a whole population is wiped out by a single spill, recovery may await recolonization or hatching of already existing resting eggs. Meanwhile, the planktivorous fish within the lake are deprived of this component of their diet. As an example, recovery of zooplankton in the pond in Barrow used in the 1970 experimental pond spill has been very slow. No *Daphnia middendorffiana* were observed there in 1975 and only a few were seen in 1977. The long-lasting effects of oil-related zooplankton mortality and the impact drastically reduced zooplankton populations may have on other organisms in arctic lakes adds urgency to research into methods to lessen and prevent oil toxicity to this important group.

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