Biological Report 15
July 1993

In Situ Toxicity Testing with Locally Collected *Daphnia*

Fish and Wildlife Service
U.S. Department of the Interior

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by

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Preface

The data from this study demonstrate a technique for in situ toxicity assessment with a resident invertebrate species. With minor adjustments, this technique should be applicable in other locations. This technique was validated in the Prudhoe Bay oil field, Alaska, in 1985 with a locally collected water flea, *Daphnia middendorffiana*. At that time, millions of gallons of wastes from reserve pits were pumped onto tundra wetlands annually. Results reported here were part of a larger study of effects of this disposal practice on water quality, of metal and hydrocarbon residues in water and sediment, and of acute and chronic toxicity to *Daphnia magna* in standard laboratory toxicity testing.

Important changes have been made in waste management practices since testing at Prudhoe Bay. Beginning in the late 1980's, the state of Alaska banned the surface disposal of reserve pit fluids. The oil industry now disposes of reserve pit fluids by injecting them into an impermeable subsurface zone. The reserve pits are maintained in a generally dry condition by rigorous fluid management to reduce seepage, overflow, and breaches of the reserve pit walls. These changes in the management of the reserve pit fluids largely eliminated new contamination of tundra wetlands from reserve pit discharges.

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Abstract. *Daphnia middendorffiana* from local tundra ponds were transplanted into five reserve pits (sumps with used drilling wastes and precipitation) at drill sites in the Prudhoe Bay oil field. Concurrently, *Daphnia* were transplanted into tundra ponds adjacent to the reserve pits ("near ponds"), into more distant but connected ponds ("distant ponds"), and into control ponds to evaluate the toxicity of the fluids along contaminant gradients. Twenty adult *Daphnia* were placed in eight waxed flow-through containers and exposed to the conditions of each test site. Two containers were removed (without replacement) at 24, 48, 72, and 96 h from each of 18 test sites. In each exposure container, the ratios of the number of dead *Daphnia* to the total number of adult *Daphnia*, the number of young *Daphnia* to the total number of adult *Daphnia*, the number of molting *Daphnia* to the total number of adult *Daphnia*, and the number of released ephippia to the total number of adult *Daphnia* were determined.

The nonparametric Friedman Rank Analysis of Variance revealed no trends in the molting or production of young or ephippia by treatment. However, the acute toxicity among reserve pits, near ponds, distant ponds, and control ponds differed significantly after 72-h ($\chi^2 = 10.20$, df = 3, $P = 0.017$) and 96-h ($\chi^2 = 9.30$, df = 3, $P = 0.026$) in situ exposures. Pairwise comparisons with the Wilcoxon's Signed Rank Test revealed significantly higher toxicity in the reserve pits than in the near ponds or control ponds ($Z = -2.023$, $P = 0.04$, both comparisons). The near ponds were also more toxic than the control ponds ($Z = -1.826$, $P = 0.068$). Immobility of *Daphnia* was most common in the reserve pits where individuals frequently floated or stuck together on the surface. These results were consistent with results from 42-day standard chronic toxicity tests with *D. magna* by other researchers at the same sites. In the in situ toxicity tests and in the standard toxicity test, fluids from the same two reserve pits were more toxic than fluids from the other three tested reserve pits. However, in situ toxicity tests revealed acute toxicity rather than chronic toxicity in reserve pits and near ponds. Also, the in situ tests revealed acute toxicity in all tested reserve pits. The results not only indicate the utility of in situ testing of invertebrate toxicity but suggest that under certain conditions in situ tests may be more sensitive than traditional laboratory toxicity tests. The increased sensitivity of *Daphnia* did not seem to be an artifact of the waxed cups used during the in situ testing; rather, it resulted either from the increased sensitivity of locally collected *D. middendorffiana* (Fischer) to the reserve pit fluids or from reduced toxicity of the fluids after shipment or during laboratory testing.

Key words: *Daphnia*, toxicity testing, contaminants, monitoring, Prudhoe Bay.

In situ toxicity testing has become increasingly popular for assessing the ecological relevance of laboratory toxicity tests (Falk 1973; Parkhurst 1987; Henry and Schoettger 1988). In situ aquatic toxicity testing, however, has been mostly conducted with fishes, not with invertebrates. In July

1985, a technique for in situ testing in the field with locally collected *Daphnia middendorffiana* (Fischer) was developed to evaluate the toxicity of drilling fluids discharged into tundra wetlands at Prudhoe Bay, Alaska. The results of this study were partially summarized and compared with results from 48-h static acute toxicity tests and 42-day chronic life cycle tests with *Daphnia magna* by Woodward et al. (1988). The descriptions of the study site and dissolved ion and hydrocarbon concentrations in water at all study sites are presented in Woodward et al. (1988).

Although the results of both types of toxicity tests were briefly described, Woodward et al. (1988) provided little detail about the in situ test method, the full range of endpoints originally measured during field testing, or the utility of using locally collected *Daphnia* as the test organism. The sorption attributes of the waxed cups used as test containers were also not described. These attributes were of potential concern because a prerequisite for toxicity testing is that the test container not release, adsorb, or absorb toxicants that modify the toxicity (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975).

Therefore, my objectives were (1) to describe the in situ toxicity tests in sufficient detail so that the technique can be repeated by other investigators, (2) to demonstrate the power of in situ testing to reveal toxicity of complex contaminant mixtures along expected contaminant gradients, (3) to evaluate the advantages and disadvantages of using waxed cups for the tests, and (4) to encourage use and further refinement of the technique with locally collected *Daphnia* and other invertebrates.

Study Area

Prudhoe Bay on the Beaufort Sea coast of Arctic Alaska is characterized by flat open expanses of wet and moist tundra vegetation dominated by the sedge *Carex aquatilis* and the grass *Arctophila fulva* (Walker 1981). Three north–south river systems and countless shallow ponds cover the area, which is underlain by permafrost. Much of the area is flooded when the snow melts, usually in June. Surface sheet flow is supplemented by water transport through a system of interconnected polygon troughs that delineate the patterned ground. Drier areas along shores of drained lake basins and on raised polygon rims are also occasionally flooded.

During the summer, the Prudhoe Bay area serves as an important nesting, rearing, molting, and feeding ground for about 150 species of shorebirds, sea birds, waterfowl, raptors, and passerines (Norton et al. 1975). Aquatic invertebrates from tundra ponds are an important food for many water birds (Pitelka 1959; Holmes 1966; Holmes and Pitelka 1968; Hilden and Vuolanto 1972; Bergman et al. 1977; Derksen et al. 1981; Connors 1983). Among these birds is the spectacled eider *Somateria fischeri,* listed as a threatened species under the Endangered Species Act of 1973 (87 Stat. 884; as amended; 16 U.S.C. 1531 et seq.) in May 1993.

The largest oil field in North America, the Prudhoe Bay oil field, is traversed by a complex system of roads and pipelines and is surrounded by additional satellite oil fields. By 1985, more than 40 drill sites had been constructed in the oil field. The roads and the drill sites were built on a thick layer of compacted gravel. Drill sites typically contained 20–32 wells, midway between the 8–16 wells drilled during early production in the late 1970's and the 64 or more wells anticipated later in production (American North Inc. 1990). From two to four reserve pits between 0.2 and 0.8 ha in surface area, 3.0 to 6.0 m deep, and dug into the gravel pad typically occurred on a drill site. The reserve pits functioned as storage pits for used drill mud, formation cuttings, workover and completion fluids, and waste oil. The pits also accumulated precipitation, primarily snow. In spring, the pits were usually full of fluids and required dewatering to avoid overtopping or breaching of the pit walls. At the time of this study, reserve pit wastes were pumped directly onto tundra wetlands adjacent to each drill site and on gravel roads and pads for dust control.

Methods and Materials

Study Sites

In situ toxicity testing was conducted from 20 July to 19 August 1985 at Drill Sites 1, 7, 12, 14, and 16 of ARCO Alaska, Inc., in Prudhoe Bay. Study sites at each drill site included (1) a reserve pit with a scheduled or an observed discharge or seepage; (2) a tundra pond receiving the reserve pit fluids through direct tundra discharge or seepage ("near pond"); and (3) a connected, more distant tundra pond ("distant pond"). Each near pond was less than 50 m from the associated reserve pit. Distant ponds were about 100–200 m from the pits and connected to the near ponds by surface water

connections. All pits and ponds were less than 2 m in average depth at the time of sampling. In situ testing was conducted at a control pond concurrent with the trial at each drill site. Control ponds resembled the other ponds in size and depth and were more than 1,000 m from any known contamination source, including road dust. Details about the locations of all sites and the discharge volumes from each reserve pit are provided by Woodward et al. (1988).

Five trials were conducted that consisted of placing daphnids in 5 reserve pits, 5 near ponds, 4 distant ponds (one drill site lacked a distant pond), and 3 control ponds. One control pond was used twice for 2 different trials, and 2 of the 5 trials were conducted concurrently so that only one control was needed. These water body types represented the range of treatments for statistical purposes.

Water Quality at the Study Sites

One-liter polyethylene containers were prerinsed at the sampling sites and used to collect surface grab samples of water from 3 locations/site to measure water quality. Dissolved oxygen was measured by a modified Winkler titration method (Hach Company 1985). Conductivity was measured with a calibrated Hach DREL/5 conductivity meter on battery power. The pH was determined with a portable Hach pH meter, standardized with 7.0 and 9.0 buffers. Total alkalinity and total hardness were measured colorimetrically by the Hach Company (1985) digital titration methods. Dissolved organic carbon was measured with a Dohrmann DC-80 carbon analyzer. Water quality determinations (except dissolved organic carbon) were made within 6 h of sample collection. An Alaska Department of Environmental Conservation field office at Prudhoe Bay (Deadhorse) served as the field laboratory.

Sites varied in temperature from 6.3 to 12.3° C (Table 1). Dissolved oxygen exceeded 8 mg/L at all sites. Conductivity ranged from 287 to 4,200 µS/cm; pH, from 8.0 to 8.7; alkalinity, from 97 to 334 mg/L; hardness, from 68 to 294 mg/L; and dissolved organic carbon, from 5.3 to 102 mg/L. Additional details on hydrocarbon and trace element contamination in water and sediment at each site are provided in Woodward et al. (1988).

Table 1. Mean water quality and temperature conditions at in situ toxicity test sites at Prudhoe Bay, Alaska, in 1985. Each value represents the mean of three measurements.

Drill site[a]	Date	Water temperature (° C)	Dissolved oxygen (mg/L)	Conductivity (µS/cm)	pH	Total alkalinity (mg/L)	Total hardness (mg/L)	Dissolved organic carbon (mg/L)
CP1	12 July	6.3	11.4	287	8.5	97	113	5.3
CP2	11 July	7.1	12.3	320	8.2	320	101	13.6
CP3	14 July	12.2	11.4	127	8.3	127	143	11.9
DP7	12 July	7.8	12.5	1,240	8.5	133	156	20.0
NP7	12 July	7.9	10.9	1,655	8.3	178	155	45.9
RP7	12 July	7.1	9.5	4,200	8.3	271	124	102.0
DP1	17 July	8.8	11.1	907	8.2	163	116	16.4
NP1	17 July	8.7	9.5	1,498	8.0	218	101	18.7
RP1	17 July	10.3	9.6	3,283	8.4	332	129	55.2
DP16	16 July	11.0	11.0	388	8.4	115	113	7.9
NP16	16 July	13.5	10.6	1,490	8.2	328	92	15.5
RP16	16 July	13.4	9.5	1,536	8.6	349	68	16.1
DP14	15 July	8.8	15.0[b]	2,533	8.3	285	222	28.1
NP14	15 July	8.0	10.3	2,933	8.3	342	294	69.7
RP14	15 July	11.4	10.1	4,100	8.4	264	266	95.3
NP12	17 July	12.0	8.5[b]	3,200	8.7	176	116	17.7
RP12	17 July	11.9	8.8[b]	4,200	8.3	137	104	75.5

[a] CP = control pond, DP = distant pond, NP = near pond, and RP = reserve pit. Numerical designations refer to actual drill site numbers.

[b] Highly variable oxygen values were obtained at these sites, possibly because of color interference.

Collection Methods

Dense swarms of *Daphnia* were collected in clean plastic 23-L fish-feed buckets from shallow portions of the control ponds; 640 similarly sized, nonmolting *Daphnia* were used for each trial. Only 1 swarm/control pond was used in each trial to maximize the probability that individuals were genetically related, if not genetically identical through parthenogenesis. The swarm was scooped up gently underwater to avoid exposing the *Daphnia* to the air. To reduce sloshing, a lid was placed on the full bucket before transporting the *Daphnia* to the field laboratory.

Flow-through Containers

Each 152-mL container consisted of two prelabeled, stacked waxed cups with cut-out bottoms separated by a 64-cm^2 square of 125-μm mesh polypropylene monofilament screen (Tetko, Inc., Elmsford, New York). The top mesh cover was secured by inverting a cut-off rim from a styrofoam cup and squeezing it over the ledge of the container (Fig. 1). When out of the treatment ponds, the entire container was temporarily nested in a third, similarly labeled, whole cold cup filled with water from either the control pond (before the test) or the test pond (after the test).

The selected mesh size was intermediate between that of standard fine-mesh plankton nets (80 μm) and coarse netting (363 μm), thereby retaining the daphnids while still permitting flow-through of small phytoplankton, protozoans, and bacteria typically consumed by daphnids.

Within 2 h of collection, *Daphnia* were randomly sorted into the flow-through containers. Then a permanent marker was used to label each container by treatment and site.

Test Organisms and Transfer Procedures

Daphnia middendorffiana was selected for the in situ tests because it is the most common species in North Slope tundra ponds. This species is well pigmented except immediately after molting (R. G. Stross, New York State University, Albany, personal communication). Adults average about 3 mm but may exceed 4 mm in length (Stross et al. 1980). *Daphnia middendorffiana* is possibly a variety of *D. pulex* (Pennak 1989), a species also found on the North Slope but distinguished by lighter pigmentation.

Because only a few neonates were present in the original samples, this life stage could not be used in the field tests. Therefore, *Daphnia* of the largest size class (2–3 mm), which predominated in our collections, were selected to reduce variability from life stage.

Sawed-off Hach Ten Sette pipets (both 10- and 1-mL tip sizes) were used to transfer 20 *Daphnia*

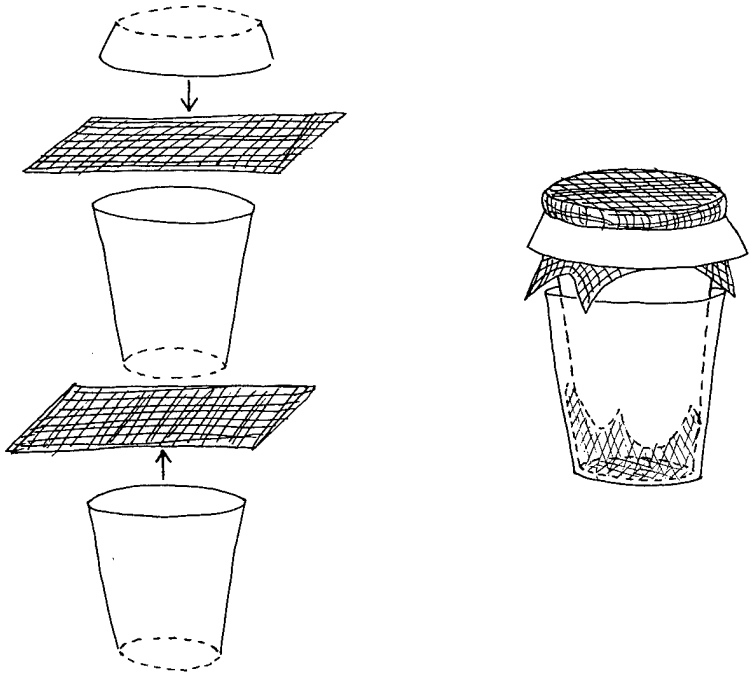

Fig. 1. Design of flow-through containers for holding *Daphnia* during in situ tests, Prudhoe Bay, Alaska, 1985. The waxed cups are separated by polypropylene mesh, which also serves as a flow-through cover. A third whole cup (not shown) holds water in the containers before they are submerged in treatment ponds.

from the collection bucket to each flow-through container. The 10-mL pipet was used for transferring groups of *Daphnia* to the cups. The pipet tip was held up vertically to the light so that the *Daphnia* could be fairly accurately counted. The 1-mL tips were used for transferring individuals; for culling *Daphnia* that were floating, laying on the bottom, or swimming erratically; and for removing all young, molts, and extraneous species from the containers.

In making the transfers, care was taken to insure that *Daphnia* for the toxicity tests were not exposed to the water surface where air bubbles could collect under the carapace and induce anomalous floating behavior. During in situ tests, usually 19 to 20 individuals were recovered from each exposure container; however, because of occasional escapees and miscounts, the number of *Daphnia* recovered per container ranged from 14 to 22 individuals. Consequently, nonparametric statistics were used to analyze the data, which were based on percent for comparability.

The containers with *Daphnia* were transported to each field location in a styrofoam beaker case. Eight containers (two containers for each 24-h time interval) were placed at each test and each control site. Only a single set of controls was used for comparison with treatments at drill sites 7 and 12 because tests at these sites were performed concurrently. The control site where *Daphnia* were tested differed from the site where they were collected except in one trial, which was conducted to evaluate the effects of environmental changes on control groups.

At the ponds, cups were attached with large stainless steel safety pins to 1.6-cm wooden dowels. Containers were placed 20–80 cm below the surface in about 1-m deep water. Care was taken to avoid stirring up sediment at sample sites. Once each cup set was attached to a dowel, the water-retaining cup with the flow-through container was slipped off to expose the bottom mesh and allow flow through. After 24, 48, 72, and 96 h, two containers were selected at random on the dowel from each treatment, nested in whole waxed cups underwater, and removed from each site for examination at the field laboratory. There was no replacement of these samples (i.e., 48-h samples contained different organisms than the 24-h samples at the same site).

Endpoints

Daphnia from each of the two containers of each treatment (reserve pit, near pond, distant pond, control pond) for a given collection period (24, 48, 72, or 96 h) were examined under a dissecting microscope in random order. Extreme care was taken in pipetting the *Daphnia* from the waxed cups into Petri dishes filled with control water to avoid exposing the organisms to the air–water interface. As in most standard laboratory toxicity tests with *Daphnia*, the acute toxicity experimental endpoint, hereafter referred to as death, was indicated by the absence of visible movement of an individual after it was prodded with a dissection microprobe or exposed to light or after the Petri dish with the individual was tapped or rotated; visible movement was defined as movement of an individual's antennae, thoracic appendages, or postabdomen (Buikema et al. 1980). A weak heartbeat was present in some immobile (dead) individuals. In addition to the ratio of dead *Daphnia* to the total number of *Daphnia*, the following additional endpoints, potentially indicative of chronic toxicity, were determined from data gathered from each container: number of young per total number of adults, number of molting adults per total number of adults, and number of ephippia released per total number of adults.

Statistical Analysis

Data were analyzed with nonparametric statistical methods to accommodate the binomial distribution expected from proportions (Conover 1971). Statistical comparisons were based on mean values of the two replicate samples removed from each treatment (reserve pit, near pond, distant pond, control pond) at each period (without replacement) when these values were available or on single values when a sample was lost. The Friedman Rank Analysis of Variance was selected to permit independent consideration of each block (trial at each drill site) in examining the effects on treatments. Analyses of variance were made on the 72- and 96-h data for each endpoint. Pairwise comparisons between reserve pits and controls, near ponds and controls, and distant ponds and controls were made with the Wilcoxon's Signed Rank Test (Steel and Torrie 1960).

Investigation of Sorption Properties of Containers

Hydrocarbon residue analysis was performed to detect aliphatic, polycyclic aromatic, and organochlorine hydrocarbons from (1) waxed cold cups filled with double-distilled, pyrogen-free water for

4 days; (2) waxed cold cups filled with double-distilled, pyrogen-free water to which a spiked hydrocarbon solution had been added for 4 days; and (3) a precleaned 1-L IChem amber glass container filled with double-distilled, pyrogen-free water to which the same spiked solution at the same dilution as above was added for 4 days.

The spiking solutions were Ultra Scientific Internal Spiking Solution certified to contain 4,000 mg/L acenapthene, chrysene, 1,4-dichlorobenzene, napthalene, perylene, and phenanthrene in methylene chloride and pure Prudhoe Bay crude oil. A 0.1-mL spike of each solution was pipetted into 1 L of double-distilled water, and 500 mL each were used in Treatments 2 and 3. Treatment 2 consisted of several waxed cups holding an equal volume, when pooled, as Treatment 3. To maintain them in a cool but not frozen condition, samples were stored and shipped together with ice packs. Samples were formulated on 4 August 1989, received by the laboratory from an air carrier on 8 September 1989, and extracted on 19 September 1989. Analysis was by gas chromatograph–mass spectrometric procedures with a detection limit of 0.004 mg/L for organochlorine compounds and of 0.001 mg/L for aliphatic and polycyclic aromatic compounds.

Results and Conclusions

Toxicity Studies

Significant differences in acute toxicity among treatments (reserve pits, near ponds, distant ponds, controls) were evident after the 72-h ($\chi^2 = 10.20$, df = 3, $P = 0.017$) and 96-h in situ exposures ($\chi^2 = 9.30$, df = 3, $P = 0.026$; Table 2). As expected from previous studies of reserve pits and associated near ponds (West and Snyder-Conn 1987; Woodward et al. 1988), the reserve pit waters had the highest 72- and 96-h acute toxicity at all five sites (Fig. 2). Acute toxicity at 96 h was significantly higher ($P \leq 0.05$) in the reserve pits than in the ponds. The death rate of the daphnids was greater in 3 of the 5 near ponds than in the control ponds. However, the differences in toxicity between the near ponds were not significant because of the lack of appreciable toxicity in the two other near ponds ($Z = -1.826$, df = 3, $P = 0.068$). There was no obvious or significant difference between the acute toxicity of the distant ponds and that of the control ponds.

The mean mortality rates of *Daphnia* in the control treatments ranged from 0 to 10% and were usually less than 5%, meeting specifications for laboratory flow-through toxicity testing (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975). Weak heartbeats were observed in most immobile individuals. This observation was possible because of the very large size of the *D. middendorffiana*. After 72 h, mobile and immobile *Daphnia* were often floating on the water's surface. These floaters frequently stuck together in groups. When pushed under the water's surface, mobile floaters did not perform the typical hop–sink swimming. However, some *Daphnia* swam well but were prone to floating and sticking. Disequilibria was also evident in many *Daphnia* in the reserve pits; some individuals whirled on the surface instead of swimming. No systematic data were collected on mobile floaters or whirlers. These phenomena were much less common in near ponds and distant ponds; immobility, sticking, and whirling were not observed in the controls.

Buikema et al. (1980) noted that surface films or suspended droplets of oil resulted in the presence of floaters. Dissolved organic carbon concentrations were highest in the reserve pits and lowest in the control ponds. At all sites except at Drill Site 14, aromatic hydrocarbon concentrations also followed expected gradients (Woodward et al. 1988).

Results of the field toxicity tests reported here were consistent with results from 42-day toxicity tests with fluids collected 1–3 weeks earlier from the same reserve pits (Woodward et al. 1988). Chronic effects were demonstrated during the 42-day toxicity tests with *D. magna* at Drill Sites 1 and 12, both at 100 and 25% concentrations of reserve pit fluids. The in situ tests also showed that reserve pits at Drill Sites 1 and 12 induced greater acute toxicity than the other study sites. Results of in situ tests were also consistent with observations of trends in the presence or absence of daphnids in the field. *Daphnia* were not observed in any reserve pit; however, they were observed in all control ponds and in near and distant ponds of Drill Site 7.

In situ results also indicated measurable toxicity in the other reserve pits and at 2 of 5 near ponds that had received reserve pit fluids. At Drill Site 1, the increased toxicity observed in our study but not in the laboratory study was at least in part the result of an oil spill that occurred in the pit between the time that the laboratory and the subsequent field study were conducted.

Table 2. Mean daily percentages of *Daphnia middendorffiana* that died or were immobilized during 96-h in situ toxicity tests conducted during July–August 1985 at five drill sites at Prudhoe Bay, Alaska. Except as noted, each value is the average of two samples from each site after a given 24-h interval. Samples collected at a particular time were not returned to the in situ site; therefore, mean values from different time intervals are independent and do not represent cumulative totals.

Drill site	Time (h)	Reserve pit	Near pond	Distant pond	Control pond
1	24	0	3	5	10
	48	74	5	8	8
	72	88	32	23	0
	96[a]	100	72	6	2
7	24	32	0	2	0
	48[a]	12	0	2	0
	72	30	8	6	0[b]
	96	60	0	0	0
12[c]	24	30	9		0
	48	14	8		0
	72	12	6[b]		0[b]
	96	80[b]	10		0
14	24	8	0	5	0
	48[a]	15[b]	8	0	0
	72	55	16	13	9
	96	58	14	10	0
16	24	2	0	2	0
	48	0	0	5	3
	72	18	25	3	5
	96	79	25	0	2

[a] Data from this time interval collected in August.
[b] Datum from a single exposure container.
[c] No distant pond available.

The increased toxicity during in situ studies at the remaining sites has several possible explanations. *Daphnia middendorffiana* may be a more sensitive species than *D. magna*. The extreme oligotrophic condition of most arctic tundra ponds (Hobbie 1980) suggests that arctic invertebrates may not adapt as readily to heavy metal or hydrocarbon contamination as species in mesotrophic or eutrophic systems where higher concentrations of trace elements and organic material are typical. Photooxidation and photolysis in nature may increase solubility and toxicity of polycyclic aromatic hydrocarbons directly (Rice et al. 1977), and such phototoxic effects may not be observed under different light conditions during laboratory studies (Giesy et al. 1983).

Woodward et al. (1988) attributed the toxicity of the reserve pit fluids primarily to the aromatic hydrocarbon content. These semi-volatile compounds may have been partially lost during shipment, from inadvertent aeration when the pit fluids were poured into static toxicity test containers, or during the toxicity tests themselves. Other factors (e.g., water quality or acclimation) may also have reduced toxicity in the laboratory toxicity tests. The temperature range of 12 ± 2° C during the laboratory toxicity test was similar to temperatures recorded at the field sites (Table 1), but other water quality factors differed.

Standard laboratory toxicity tests with *D. magna* revealed no acute toxicity in 48-h tests of reserve pit fluids. The in situ testing also showed no appreciable increase in mortality or immobilization until after 48 h. This finding is consistent with oil-induced toxicity in *D. middendorffiana*; O'Brien (1978) found that significant toxicity did not occur until after 48 h of exposure to Prudhoe Bay crude oil and reached a maximum at 120 h, at

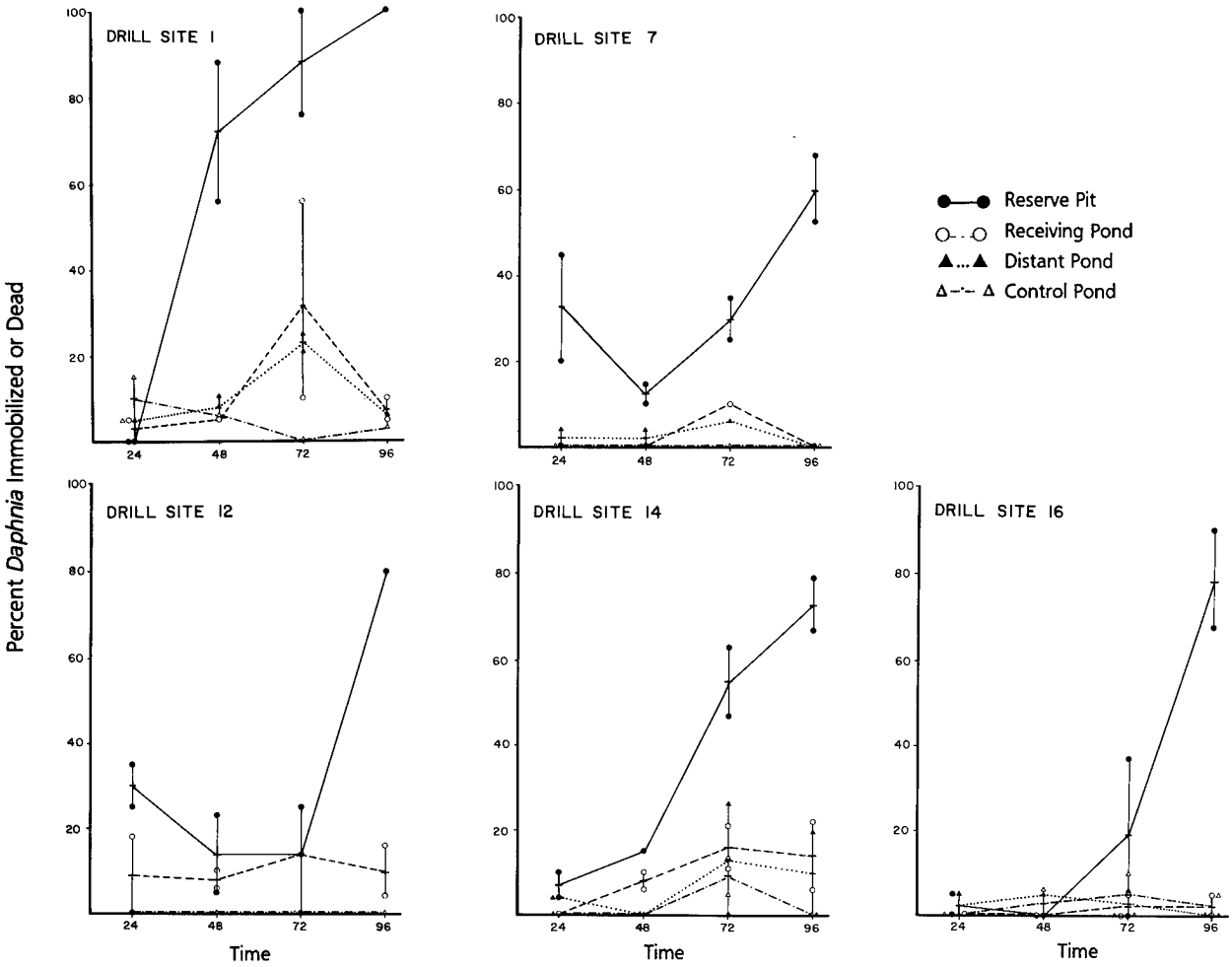

Fig. 2. Mean daily percentages of *Daphnia middendorffiana* that died or were immobilized during in situ tests in reserve pits, near ponds receiving reserve pit effluent, more distant ponds, and control ponds at Prudhoe Bay, Alaska, 1985. *Vertical lines* connect replicate sample values. *Lines* link means per time interval.

which time control mortality was also first noted. In this study, excess *Daphnia*, retained in the lab during the 96-h field tests without food and at high density, remained vigorous swimmers and did not float or whirl. This indicates that nutritional deficiencies did not control mortality or impair swimming during field tests.

Influence of Test Site Conditions

Reserve pit fluids are complex effluents that vary considerably from pit to pit (West and Snyder-Conn 1987; Woodward et al. 1988). Their complexity derives from the variety of drilling muds, drill cuttings, completion fluids, and formation hydrocarbons and with precipitation in each pit. Some pit fluids tested in this study contained relatively low concentrations of metals or hydrocarbons, whereas others contained much higher concentrations (Woodward et al. 1988). Unusual and highly variable water quality also occurred in some of the reserve pits, especially in association with high concentrations of metals, such as barium, lead, chromium, and zinc. High metal concentrations in reserve pits correlated with high turbidity, conductivity, hardness, pH, and alkalinity (West and Snyder-Conn 1987; Woodward et al. 1988). In standard laboratory toxicity testing, the use of stock dilution water reduces variations in water quality among test solutions. Frequently, water quality factors such as pH, hardness, and suspended solids are also directly controlled (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975; Buikema et al. 1980). Another limitation of laboratory toxicity tests is that aromatic hydrocarbons, found in many Prudhoe Bay reserve pit fluids (West and Snyder-Conn

1987; Woodward et al. 1988), may be lost during shipment of the water samples to the laboratory, during the holding period before testing, and during testing.

During the in situ testing, production of young, molts, and ephippia was highly variable within and among sites (Tables 3–5). This variation was probably due in part to differences in temperature and water quality during the tests, which were conducted in July and August. In August, the water temperatures were cooler, young ceased to be produced, and overwintering egg (ephippia) production ensued. Probably less variability in production among sites would have been observed if all the 96-h tests had been conducted concurrently in either July or August. Consequently, data on the production of young and ephippia could not be compared among any of the discontinuous test series. Nevertheless, variations in temperature, conductivity, nutritional status, and other environmental conditions were probably the most important controlling factors for production of young (Cowgill et al. 1985; Gersich et al. 1985). The production of ephippia seemed to correlate strongly with season rather than with short-term exposure to drilling fluids.

The use of 20 *Daphnia*/152-mL cup was feasible because the experiments were flow through water temperatures at all sites were very cold, and dissolved oxygen values were above 8 mg/L (Table 1). However, a reduced loading of 10 *Daphnia*/cup may be required in other situations (e.g., if anomalous mortality occurs in controls, if dissolved oxygen is low, or if water temperatures at test sites are high).

Table 3. Mean daily percentages of *Daphnia middendorffiana* molting during 96-h in situ toxicity tests conducted in July–August 1985 at five drill sites at Prudhoe Bay, Alaska. Except as noted, each value is the average of two samples from each site after a given 24-h interval. Samples collected at a particular time were not returned to the in situ site; therefore, mean values from different time intervals are independent and do not represent cumulative totals.

Drill site	Time (h)	Reserve pit	Near pond	Distant pond	Control pond
1	24	5	4	5	8
	48	16	18	16	28
	72	32	35	48	40
	96[a]	0	15	19	24
7	24	5	10	8	5
	48[a]	5	24	12	6
	72	22	11	18	6[b]
	96	29	12	31	31
12[c]	24	5	8		5
	48	18	15		6
	72	12	6[b]		6[b]
	96	25[b]	62		31
14	24	28	35	20	38
	48[a]	25[b]	25	22	20
	72	71	82	58	86
	96	68[b]	94	74	78
16	24	31	28	18	5
	48	10	20	15[b]	24
	72	32[b]	34	28	18
	96	68	32	20	20

[a] Data from this time interval collected in August.
[b] Datum from a single exposure container.
[c] No distant pond available.

Table 4. Mean daily percentages of live young *Daphnia middendorffiana* produced during 96-h in situ toxicity tests in July 1985 at two drill sites at Prudhoe Bay, Alaska.[a] Each value is the average of two samples from each site after a given 24-h interval. Samples collected at a particular time were not returned to the in situ site; therefore, mean values from different time intervals are independent and do not represent cumulative totals.

Drill site	Time (h)	Reserve pit	Near pond	Distant pond	Control pond
1	24	2	28	0	25
	48	19	49	58	70
	72	75	72	136	66
14	24	58	108	72	84
	72	118	266	108	215
	96	176	286	180	248

[a] Only toxicity tests with complete data sets collected in July are presented. Data sets containing August samples are omitted because few or no young were produced in these tests.

Table 5. Mean daily percentages of *Daphnia middendorffiana* ephippia produced during 96-h in situ toxicity tests in August 1985 at three drill sites at Prudhoe Bay, Alaska.[a] Percentages represent number of ephippia divided by the number of present adults. Except as noted, each value is the average of two samples from each site after a given 24-h interval. Samples collected at a particular time were not returned to the in situ site; therefore, mean values from different time intervals are independent and do not represent cumulative totals.

Drill site	Time (h)	Reserve pit	Near pond	Distant pond	Control pond
7	24	0	2	2	0
	48	0	6	5	6
	72	0	8	3	0[b]
	96	2	8	5	5
12[c]	24	0	9		0
	48	2	8		6
	72	2	5[b]		0[b]
	96	25[b]	13		5
16	72	10	10	18	10
	96	8	12	29	6

[a] Tests at Drill Sites 7, 12, and 16 were completed in August. Only data from August are presented because no ephippia were noted during tests in July.

[b] Datum from a single exposure container.

[c] No distant pond available.

Ten individuals/cup could also be more accurately counted, but additional replicates may be required to achieve the same level of confidence in the results, depending on statistical test.

All large *Daphnia* selected in this study were probably similar in age because only one overwintering stage of *Daphnia* occurs each year on the North Slope of Alaska and synchronization confines production of the resulting adults to a 36-h period in midsummer (Edmondson 1955; Stross et al. 1980). This may have strongly reduced the variation that may be observed in more temperate locations if multigenerational swarms are used for the in situ test.

Influence of Sorption from Waxed Paper Cups

The use of waxed cold cups (drinking water cups) offered several advantages, including cleanliness,

availability, low cost, and transportability. The round shape was highly desirable for reducing accidental crushing of the *Daphnia*. The container design also avoided problems of air retention that occur in some other test-chamber designs (O'Brien 1978). However, the sorption properties of the waxed cups (Lily cold cups) and their potential effects on toxicity were unknown before testing.

No organochlorine pesticides or PCBs were detected in any sample that was processed with gas chromatograph–mass spectrometric procedures. Biphenyl was detected in the spiked solution in both the waxed cups (0.007 mg/L) and the amber glass jar (0.014 mg/L) but not in the reference solution (Treatment 1), indicating potential spike contamination. No cyclic alkanes were detected in any treatment (Tables 6 and 7).

The waxed cups did not release measurable concentrations of aliphatic or polycyclic aromatic compounds because none of these compounds was detected in distilled water held in the cups (Treatment 1). This finding was also supported by high survival of *Daphnia* during in situ exposures at control sites. Therefore, any positive toxicity results could not be attributed to release of the materials from the container under natural oligotrophic conditions. However, interactive effects on the waxed cups' hydrocarbons from the presence of contaminants in the test water cannot be ruled out entirely.

The results suggest that, unlike similarly spiked borosilicate glass containers (Treatment 3), the waxed cups may absorb or adsorb organic compounds (Treatment 2), but results are not definitive. The average aliphatic retention of the spike solution in the water of waxed cups was 50% of the aliphatic retention rate observed in the glass container. The retention rate in the waxed cups was surprisingly constant among alkanes. Among the aromatic compounds, only five compounds occurred in concentrations greater than 10 times the detection limit. Below this threshold, values are qualitative. The average retention of those five aromatics in the waxed cups was 54.2% of that in the amber glass jar. Again, retention rates were surprisingly consistent among compounds except fluorene. Because the contents of several waxed cups were pooled to yield a sample volume equal to that in the glass bottle, it is possible that volatility or physical loss during pouring from the cups into the glass container submitted for analysis accounted for much of this loss. If sorption is the main cause of the differences, toxicity could be potentially reduced in the cups, but flow through of new water during the exposure period would tend to offset these losses.

Conclusions

In situ toxicity testing with locally collected *Daphnia* proved to be a quick, inexpensive, effective technique for screening in the field. If sufficient *Daphnia* are unavailable, the technique could probably be adapted to other filter feeders, such as fairy shrimp, copepods, amphipods, and certain aquatic insects. When money for analysis is scarce, laboratory facilities are limited, or complex or unknown contaminant problems are suspected, this technique seems to be appropriate for evaluating the response of the crustacean community. When gradients in pollutant concentrations exist, the technique could also be used to determine lethal concentration or compliance with mixing zone requirements. Another potential application of in situ testing is to determine the effects of fluctuating or episodic pollution for which conventional exposure tests are clearly inadequate (Seager and Maltby 1989). Finally, the technique is useful for validation in the field of more rigorous single-species laboratory toxicity tests.

Table 6. Aliphatic hydrocarbons (milligrams per liter) recovered in laboratory sorption experiment on 4 August 1989.[a]

Treatment[b]	C12	C13	C14	C15	C16	C17	Pristane	C18	Phytane	C19	C20
1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2	0.039	0.041	0.042	0.041	0.035	0.027	0.018	0.024	0.012	0.035	0.032
3	0.070	0.079	0.084	0.074	0.062	0.069	0.050	0.052	0.034	0.053	0.055

[a] C12 = n-dodecane, C13 = n-tridecane, C14 = n-tetradecane, C15 = n-pentadecane, C16 = hexadecane, C17 = n-heptadecane, C18 = n-octadecane, C19 = n-nonadecane, C20 = n-eicosane.

[b] Treatment 1 = double-distilled water in waxed cups (no spike added), Treatment 2 = hydrocarbon-spiked solution in waxed cups, and Treatment 3 = hydrocarbon-spiked solution (same concentration as Treatment 2) in amber glass container.

Table 7. Polycyclic aromatic hydrocarbons (milligrams per liter) recovered in a laboratory sorption experiment on 4 August 1989.[a] Except for perylene (in original spike), compounds detected at or below the detection limit of 0.001 mg/L are not reported.

Treatment[b]	NAP	ACNA	ACNT	F	PA	MPA	FL	P	BAA	CH	BEP	PR	BPR
1	<0.005	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2	0.157	0.001	<0.001	<0.001	0.008	0.008	<0.001	<0.001	<0.001	0.002	<0.001	<0.001	<0.001
3	1.290	0.001	0.001	0.009	0.018	0.013	0.001	0.001	0.001	0.003	0.001	<0.001	<0.001

[a] NAP = total naphthalenes (naphthalene + 1-methylnaphthalene + 2-methylnaphthalene + 2,6-dimethylnaphthalene + 2,3,4- trimethylnaphthalene), ACNA = acenaphthylene, ACNT = acenaphthene, F = fluorene, PA = phenanthrene, MPA = methylphenanthrene, FL = fluoranthene, P = pyrene, BAA = benzo(a)anthracene, CH = chrysene, BEP = benzo(e)pyrene, PR = perylene, and BPR = benzo(ghi)perylene.

[b] Treatment 1 = double-distilled water in waxed cups (no spike added), Treatment 2 = hydrocarbon-spiked solution in waxed cups, and Treatment 3 = hydrocarbon-spiked solution (same concentration as Treatment 2) in amber glass container.

Acknowledgments

This study was part of a larger study with D. Woodward of the U.S. Fish and Wildlife Service. S. James volunteered technical assistance with toxicity tests and provided camaraderie during the study. L. Dietrick of the Alaska Department of Environmental Conservation generously provided office space that served as the field laboratory in Prudhoe Bay. C. Moitoret assisted with field collections, and D. Thomas provided statistical advice for data analysis. J. Fairchild, L. Cleveland, C. Ingersoll, J. Huckins, H. Metsker, J. Reid, and E. Robinson-Wilson provided valuable technical and editorial comments on the original draft of this manuscript. The manuscript was also reviewed by S. Elliot and P. Sousa. D. Kehrle provided the artistry for the cover.

Cited Literature

American North Inc. [sic] 1990. Oil and gas exploration, development, and production in the Arctic National Wildlife Refuge: waste management issues. Pages 1–150 *in* Oil and gas waste management issues and recommendations for the Arctic National Wildlife Refuge. Alaska Department of Environmental Conservation Division of Environmental Quality Report, Juneau, Alaska.

Bergman, R. D., R. L. Howard, K. F. Abraham, and M. W. Weller. 1977. Water birds and their wetlands resource in relation to oil development at Storkersen Point, Alaska. U.S. Fish and Wildlife Service Resource Publication 129. 38 pp.

Buikema, A. L., Jr., J. G. Geiger, and D. R. Lee. 1980. *Daphnia* toxicity tests. Pages 48–69 *in* A. L. Buikema and J. Cairns, editors. Aquatic invertebrate bioassays. American Society for Testing and Materials Special Technical Publication 715.

Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. U.S. Environmental Protection Agency EPA-660/3-75-009. 61 pp.

Connors, P. G. 1983. Effects of food resource levels on tundra bird populations. Pages 1–50 *in* Prudhoe Bay Waterflood Project Environmental Monitoring Program 1982. Volume 4, chapter 7. Final report. U.S. Army Corps of Engineers, Alaska District, Anchorage, Alaska.

Conover, W. J. 1971. Practical nonparametric statistics. John Wiley & Sons, Inc., New York. 462 pp.

Cowgill, U. M., D. L. Hopkins, S. L. Applegath, I. T. Takahashi, S. D. Brooks, and D. P. Milazzo. 1985. Brood size and neonate weight of *Daphnia magna* produced by nine diets. Pages 233–244 *in* R. C. Bahner and D. J. Hansen, editors. Aquatic toxicology and hazard assessment: eighth symposium. American Society for Testing and Materials Special Technical Publication 891.

Derksen, D. V., T. C. Rothe, and W. D. Eldridge. 1981. Use of wetland habitats by birds in the National Petroleum Reserve—Alaska. U.S. Fish and Wildlife Service Resource Publication 141. 27 pp.

Edmondson, W. T. 1955. The seasonal life history of *Daphnia* in an arctic lake. Ecology 36:439–455.

Falk, M. R. 1973. Simple apparatus for conducting acute toxicity bioassays under field conditions. Water Research 7:821–822.

Gersich, F. M., D. L. Hopkins, S. L. Applegath, C. G. Mendoza, and D. P. Milazzo. 1985. The sensitivity of chronic endpoints used in *Daphnia magna* Straus lifecycle tests. Pages 242–252 *in* R. C. Bahner and D. J. Hansen, editors. Aquatic toxicology and hazard assessment: eighth symposium. American Society for Testing and Materials Special Technical Publication 891.

Giesy, J. P., S. M. Bartell, P. F. Landrum, G. L. Leversee, and J. W. Bowling. 1983. Project summary: fates and biological effects of polycyclic aromatic hydrocarbons in aquatic systems. U.S. Environmental Protection Agency EPA-600/S3-83-053. 5 pp.

Hach Company. 1985. Water analysis handbook. Hach Co., Loveland, Colo. v.p.

Henry, M. G., and R. A. Schoettger. 1988. Aquatic toxicity test methodologies: an update for the 1980's. Pages 137–145 *in* R. C. Ryans, editor. Protection of river basins, lakes, and estuaries: fifteen years of cooperation toward solving environmental problems in the USSR and USA. American Fisheries Society, Bethesda, Md.

Hilden, O., and S. Vuolanto. 1972. Breeding biology of the red-necked phalarope, *Phalaropus lobatus* in Finland. Ornis Fennica 49:57–85.

Hobbie, J. E., editor. 1980. Limnology of tundra ponds: Barrow, Alaska. US/IBP Synthesis Series 13. 514 pp.

Holmes, R. T. 1966. Feeding ecology of the red-backed sandpiper (*Calidris alpina*) in arctic Alaska. Ecology 47:32–45.

Holmes, R. T., and F. A. Pitelka. 1968. Food overlap among coexisting sandpipers on northern Alaska tundra. Systematic Zoology 17:305–318.

Miller, M. C., R. T. Prentki, and R. J. Barsdate. 1980. Physics. Pages 51–75 *in* J. E. Hobbie, editor. Limnology of tundra ponds. US/IBP Synthesis Series 13. 514 pp.

Norton, D. W., I. W. Alies, and J. A. Curatolo. 1975. Ecological relationships of the inland tundra avifauna near Prudhoe Bay, Alaska. Pages 124–133 *in* J. Brown, editor. Ecological investigations on the Tudra Biome in the Prudhoe Bay region, Alaska. Biological Paper, University of Alaska, Special Report 2.

O'Brien, W. J. 1978. Toxicity of Prudhoe Bay crude oil to Alaskan arctic zooplankton. Arctic 31:219–228.

Parkhurst, B. R. 1987. A comparison of laboratory and in situ bioassays for evaluating the toxicity of acidic waters to brook trout. Ph.D. dissertation, University of Wyoming, Laramie. 131 pp.

Pennak, R. W. 1989. Fresh-water invertebrates of the United States. Protozoa to Mollusca. 3rd edition. John Wiley & Sons, Inc., New York. 385 pp.

Pitelka, F. A. 1959. Numbers, breeding schedule, and territoriality in pectoral sandpipers of northern Alaska. Condor 61:233–264.

Rice, S. D., J. W. Short, and J. F. Karinen. 1977. Comparative oil toxicity and comparative animal sensitivity. Pages 78–94 *in* D. A. Wolfe, editor. Fate and effects of petroleum hydrocarbons in marine organisms and ecosystems: proceedings. Pergamon Press, Inc., New York. 478 pp.

Seager, J., and L. Maltby. 1989. Assessing the impact of episodic pollution. Hydrobiologia 188/189:633–640.

Steel, R. G. D., and J. H. Torrie. 1960. Principles and procedures of statistics with special reference to the biological sciences. McGraw-Hill Book Co., New York. 481 pp.

Stross, R. G., M. C. Miller, and R. J. Daley. 1980. Zooplankton. Pages 251–296 *in* J. E. Hobbie, editor. Limnology of tundra ponds: Barrow, Alaska. US/IBP Synthesis Series 13.

Walker, D. A. 1981. The vegetation and environmental gradients of the Prudhoe Bay region, Alaska. M.S. thesis, University of Colorado, Boulder. 484 pp.

West, R. L., and E. Snyder-Conn. 1987. Effects of Prudhoe Bay reserve pit fluids on water quality and macroinvertebrates of arctic tundra ponds in Alaska. U.S. Fish and Wildlife Service Biological Report 87(7). 48 pp.

Woodward, D. F., E. Snyder-Conn, R. G. Riley, and T. R. Garland. 1988. Drilling fluids and the arctic tundra of Alaska: assessing contamination of wetlands habitat and the toxicity to aquatic invertebrates and fish. Archives of Environmental Contamination and Toxicology 17:683–697.

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